



# Thermo Scientific Capillary High-Pressure Ion Chromatography

simplifying IC while increasing resolution and speed

# Capillary Ion Chromatography

Bringing a new level of speed and resolution

Capillary IC takes performance and ease-of-use to a whole new level, simplifying ion analysis while increasing speed of analysis. Thermo Scientific™ Dionex™ Capillary HPIC™ High-Pressure Ion Chromatographs provide true on-request 24/7 uptime with no equilibration necessary, allowing the system to be always ready, delivering better results.

The Thermo Scientific Dionex ICS-5000+ and the Dionex ICS-4000 Systems are our latest innovations in capillary ion chromatography. When combined with Thermo Scientific™ Dionex™ IonPac™ 4µm particle-size capillary columns, these systems can facilitate separations up to 2 times faster than 2 mm or 4 mm columns.

Dionex ICS-5000+ and Dionex ICS-4000 High-Pressure Ion Chromatography Systems





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# Benefits of Capillary Ion Chromatography

By scaling down column size, injection volumes, and flow rates by a factor of 10 to 100, Capillary IC provides excellent eluent economy, small sample requirements, and high mass sensitivity.

## Always Ready

With capillary IC, only 15 mL of water a day are consumed, which translates to 5.2 L a year. As a result, the system can operate with continuous eluent flow, thereby eliminating the need to wait for equilibration, and providing a true on-request system.

## Lower Cost of Ownership

When systems are operated as a Reagent-Free™ Ion Chromatograph (RFIC™), eluent generation cartridges last for 18 months. Maintenance costs are minimal because the pump only dispenses water, resulting in less wear on piston seals.

## Optimum Balance Between Resolution and Throughput

With the ability to operate a capillary system continuously up to 5000 psi, high resolution and fast run times are achievable using new 4 µm particle-size capillary columns. Dionex IonPac 4 µm particle-size columns provide fast separations when using short (150 mm long) columns, and high resolution when using standard length (250 mm long) columns.

## Improved Sensitivity with 2D-IC

Two dimensional IC (2D-IC) with a standard bore column in the first dimension (i.d. 4 mm) and with a capillary column (i.d. 0.4 mm) in the second dimension permits low detection limits in the ng/L range.

Table 1. Comparison of analytical versus capillary ion chromatography operating parameters.

	Analytical	Capillary
Column i.d.	4 mm	0.4 mm
Flow Rate	1.0 mL/min	0.010 mL/min
Injection Volume	40 µL	0.4 µL
Eluent Consumption/Waste Generated	43.2 L/month	0.432 L/month
EGC Cartridge Lifetime (at 75 mM)	28 Days	18 Months
Mass Detection Limits	7000 fg	70 fg

# Capillary High-Pressure Ion Chromatography Systems

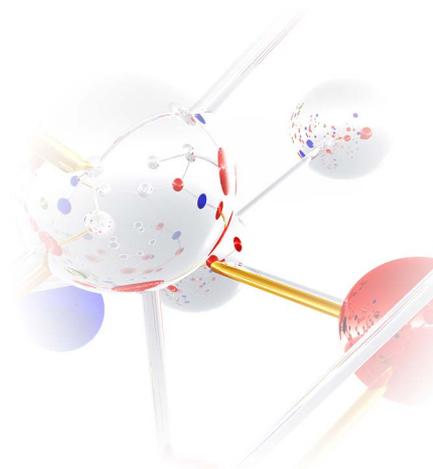
The Dionex ICS-5000+ HPIC and the Dionex ICS-4000 HPIC systems are the next generation of ion chromatography systems, offering the ability to operate as an RFIC system at up to 5000 psi. The high-pressure capability offers the flexibility to use small-particle columns for higher resolution without sacrificing speed, or higher flow rates with standard length or shorter (150 mm) columns for high throughput. As a capillary system, the reduced eluent flow rate reduces eluent consumption and produces less waste. The Thermo Scientific™ Dionex™ IC Cube™ redefines ease-of-use by color-coding fittings and reducing the number of connections in the system; all with an array of detectors for our most flexible systems.

## Dionex ICS-5000+ HPIC System

The Dionex ICS-5000+ HPIC is the world's first universal high-pressure ion chromatography system. Developed with flexibility, modularity, and ease-of-use in mind, the Dionex ICS-5000+ HPIC system combines the highest sensitivity with convenience. With the ability to analyze samples at capillary, microbore, or standard flow rates (or any combination of two, in a dual system) at up to 5000 psi, the system is our most adaptable IC system. The wide variety of Dionex ICS-5000+ modules allows you to configure an ion chromatography system designed specifically to provide the analytical solutions you need.

## Dionex ICS-4000 HPIC System

The Dionex ICS-4000 Capillary HPIC system is our first dedicated capillary ion chromatography system. The Dionex ICS-4000 Capillary HPIC system delivers best-in-class sensitivity, from routine analyses to your most demanding application challenges. The high-pressure capability brings a new level of resolution and speed to ion chromatography, while the integrated design of the Dionex ICS-4000 HPIC system simplifies your workflow and increases your analytical efficiency and productivity. Additionally, with an array of detectors and options, the Dionex ICS-4000 HPIC system adds up to be an incredibly versatile system.



## Best Practices

With the low flow rates and small volumes used in capillary IC, the number of connectors, void volumes, and the tubing lengths between modules must be minimized during installation. To achieve good chromatography by capillary IC, it is critical to:

1. Use precision-cut tubing and appropriate fittings for all connections.
2. Minimize the void volume between tubing and fittings.
3. Flush and hydrate all consumables and cartridges according to the respective device and system manuals.
4. Flush air bubbles from the system initially and after any change to the system.
5. Keep the capillary IC system running to maintain stability.
6. If the system is shut down, open the prime valve on the pump to relieve pressure and minimize residual flow.

### Maximizing the benefits of Capillary IC

The above best practices will help ensure that good chromatographic results are obtained using the low volumes typical of capillary IC systems. Full details and additional instructions can be found in [Thermo Scientific Technical Note 113: Practical Guidance for Using Capillary Ion Chromatography](#).



# Flow Path

Capillary RFIC systems have many similarities to standard bore RFIC systems. In RFIC systems, a dual piston pump delivers deionized water to an Eluent Generator Cartridge (EGC), where the eluent is electrolytically generated in-line to the injection valve. At the valve, the autosampler introduces the sample through a sample loop (or concentrator column) to the ion-exchange column. The ions are then separated and eluted into the suppressor where the background conductivity is reduced to nearly zero, and the strong acid or base forms of the ions are detected by the conductivity detector.

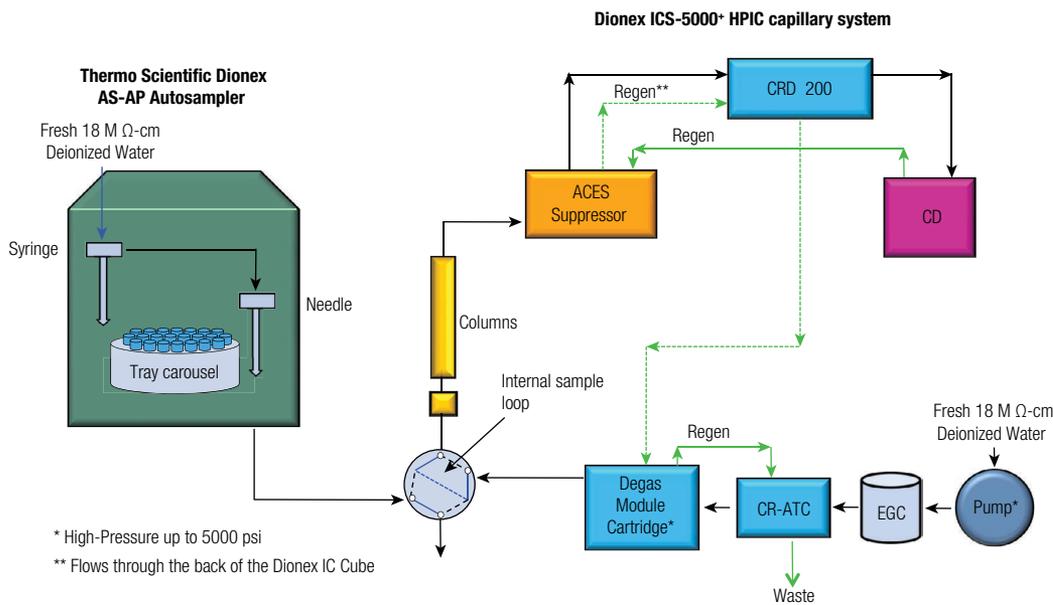


Figure 1. Flow diagram for a Dionex capillary IC System.

## The Dionex IC Cube

Capillary IC uses 0.001 to 0.100 mL/min flow rates. The Dionex IC Cube was designed to minimize the dead volume in the flow path (Figure 2). The Dionex IC Cube contains the EG Degas, Columns, Column Tray, Capillary Electrolytic Suppressor, and Thermo Scientific Dionex CRD 200 (Capillary) carbonate removal device cartridges, plus the injection valve.

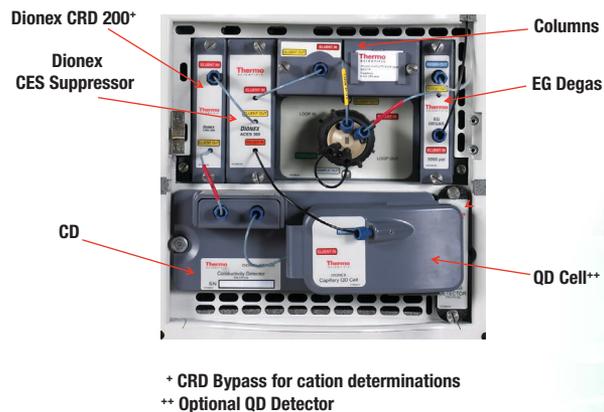


Figure 2. Components of the Dionex IC Cube module shown with the Dionex QD and CD detectors.

## Small Particles, Big Benefits

Chromatographic separations using packed columns benefit from a high number of theoretical plates per column. The number of theoretical plates can be increased by packing smaller resin particles into the columns. Typically ion chromatography columns use resin particles ranging from 7–9  $\mu\text{m}$  in diameter. Recent developments in resin technology have allowed the use of 4  $\mu\text{m}$  resin particles in ion exchange columns.

The benefits of columns packed with smaller particles include:

- More efficient peaks
- Better resolution
- Faster run times
- Easier integration
- More reliable results



4  $\mu\text{m}$  particle-size columns are available in 4 mm, 2 mm, and capillary formats and offer significant benefits in higher resolution and faster separations.

In addition, the length of the column can be optimized for desired results. Shorter, 150 mm length columns can be used at higher flow rates to increase productivity without sacrificing performance. Longer, 250 mm length columns are utilized for higher resolution separations of complex sample matrices. As a result, these columns allow one to choose the optimum balance between resolution and throughput.

Figure 3 shows an example for a fast separation of inorganic cations and biogenic amines in less than 9 minutes, using a capillary Dionex IonPac CS19-4 $\mu\text{m}$  column. To achieve these fast run times, the flow rate was increased from the standard of 0.010 mL/min to 0.020 mL/min and a steep gradient was applied.

Peaks:	
1. Lithium	0.05 mg/L
2. Sodium	0.2
3. Ammonium	0.25
4. Potassium	0.5
5. Magnesium	0.25
6. Calcium	0.5
7. Impurity	
8. Putrescine	7.5
9. Cadaverine	4.5
10. Histamine	6.5
11. Agmatine	5.0
12. Spermine	3.0
13. Spermidine	1.5

Note: Concentrations are approximate

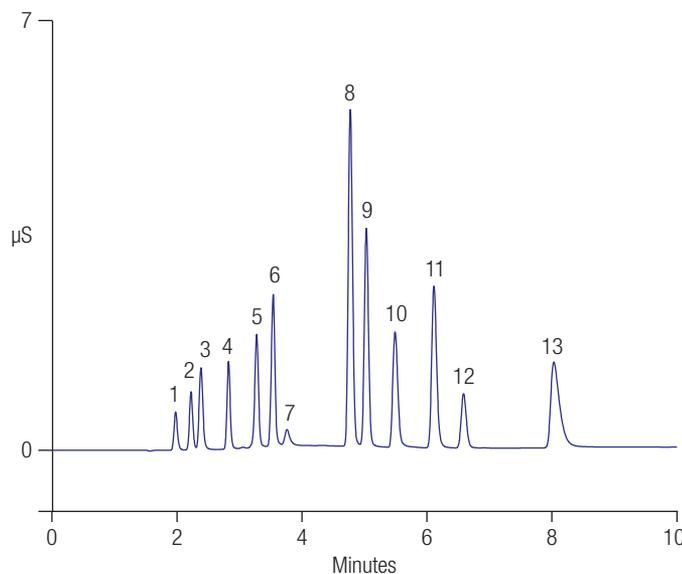


Figure 3. Fast separation of six inorganic cations and biogenic amines using the capillary Dionex IonPac CS19-4 $\mu\text{m}$  column.

# Capillary Electrolytic Eluent Generation

An electrolytic eluent generator in a capillary format provides an ideal eluent generation and delivery platform for both isocratic (Figure 4) and gradient (Figure 5) capillary separations for several reasons:

- Provides reliable and convenient eluent sources and eliminates the errors associated with manual eluent preparation.
- Electrolytic eluent generators are capable of providing precise and accurate gradients through precise current and flow rate controls.
- At capillary flow rates eluent concentrations up to 200 mM can be generated, enabling faster elution of highly retained ions.
- With RFIC, only water is delivered through the pump, which prolongs pump seals and reduces maintenance costs.

Thermo Scientific Dionex capillary RFIC systems are capable of highly reproducible separation of analytes under gradient elution by minimizing the error that comes with manually preparing eluents and eliminating contamination from the environment (e.g., carbon dioxide) as shown in Figure 5.

## Overlay of 30 consecutive runs

Retention time RSD (n = 30):  
0.060% (bromide) to 0.091% (fluoride)  
Peak area RSD (n = 30):  
0.24% (sulfate) to 0.50% (fluoride)

Peaks:

1. Fluoride
2. Bromate
3. Chloride
4. Nitrite
5. Chlorate
6. Bromide
7. Nitrate
8. Sulfate

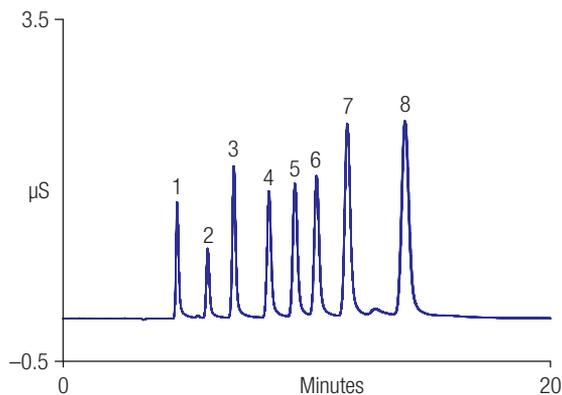


Figure 4. Isocratic separation of common anions obtained using a capillary Dionex IonPac AS19 column.

Peaks:

- |             |              |                 |
|-------------|--------------|-----------------|
| 1. Fluoride | 8. Chlorate  | 15. Iodide      |
| 2. Acetate  | 9. Bromide   | 16. Chromate    |
| 3. Formate  | 10. Nitrate  | 17. Thiosulfate |
| 4. Chlorite | 11. Sulfate  | 18. Phosphate   |
| 5. Bromate  | 12. Malonate | 19. Fumarate    |
| 6. Chloride | 13. Selenate | 20. Arsenate    |
| 7. Nitrite  | 14. Oxalate  | 21. Thiocyanate |
|             |              | 22. Perchlorate |

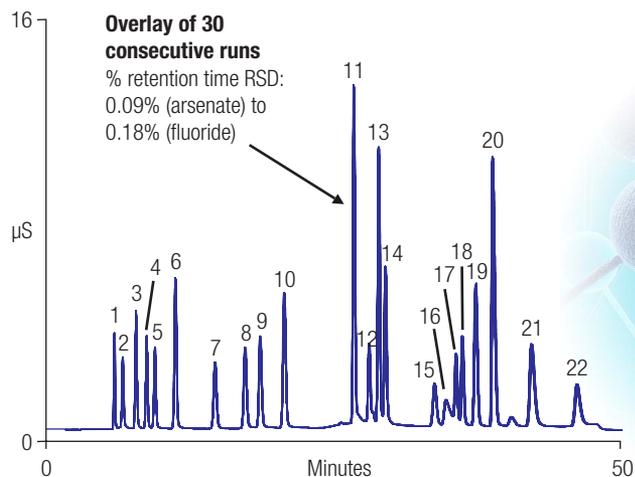


Figure 5. Gradient separation of 22 anions using a capillary Dionex IonPac AS19 column.

## Enhanced Determination of Trace Analytes

Capillary RFIC systems provide improved determination of analytes at trace levels because measurements can be accomplished using relatively small volumes. One approach is to perform a large-volume direct injection, which is suitable for samples with low levels of matrix ions. A 10  $\mu\text{L}$  injection onto a 0.4 mm i.d. column in a capillary IC system is equivalent to a 1000  $\mu\text{L}$  injection onto a 4 mm i.d. column. Another approach is loading a 250  $\mu\text{L}$  sample onto a capillary concentrator rather than by loading a 25 mL sample onto a 4 mm concentrator. Therefore, capillary IC systems can offer significant benefits in trace analysis, especially in applications where sample volumes are limited.

Figure 6 demonstrates the separation of inorganic anions at trace concentrations using a capillary Dionex IonPac AS19 column. With a 10  $\mu\text{L}$  injection, a capillary RFIC system is capable of determining the target anions at concentrations ranging from 0.2  $\mu\text{g/L}$  to 1.0  $\mu\text{g/L}$  with excellent signal-to-noise ratios.

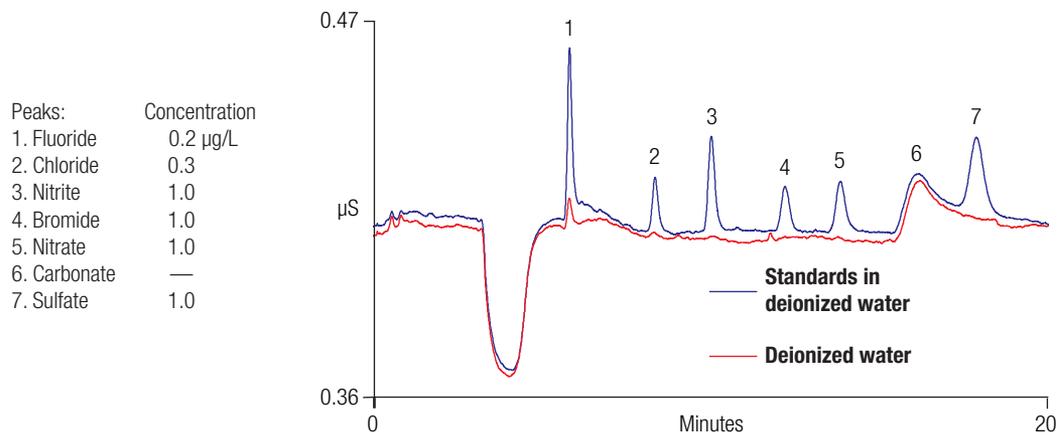


Figure 6. Separation of inorganic anions at trace concentrations using a capillary Dionex IonPac AS19 column with a 10  $\mu\text{L}$  injection.

# Capillary Electrolytic Suppressor

The Thermo Scientific™ Dionex™ CES™ 300 Capillary Electrolytic Suppressors are optimized for eluent flow rates typically seen in capillary systems (5–30  $\mu\text{L}/\text{min}$ ). When used for anion analysis, the Thermo Scientific™ Dionex™ ACES™ 300 Anion Capillary Electrolytic Suppressor converts highly conductive hydroxide-based eluents into pure water, reducing the baseline conductivity. While suppressing the eluent, the Dionex ACES 300 suppressor also converts the analytes into their more conductive hydronium (acid) form, increasing their sensitivity under conductivity detection. Likewise, when used for cation analysis, the Thermo Scientific™ Dionex™ CCES™ 300 Cation Capillary Electrolytic Suppressor converts highly conductive methanesulfonic acid (MSA) eluents into pure water; simultaneously, the analytes are converted to their more conductive hydroxide form, increasing their sensitivity.

## Dionex CES 300 Suppressor Technology

The Dionex CES suppressor uses a three-chamber design to minimize dead volume while maximizing suppression capacity and reducing noise. The eluent chamber is comprised of an ion-exchange capillary membrane that facilitates the efficient exchange of the eluent counter ions for regenerant ions. The regenerant chambers are divided into ion-exchange and electrode chambers. Regenerant first passes through the ion-exchange chamber, which is filled with a bed of ion exchange resin; the ion exchange capillary membrane is coiled in this bed. The regenerant bed is an ion exchange resin in the opposite form as the eluent. It is this bed of resin that provides the regenerant ions for the capillary membrane eluent chamber. There are two electrode chambers that are separated from the ion-exchange chamber by a pair of ion exchange membranes. Regenerant, after passing through the ion-exchange chamber, passes through the cathode and anode chambers serially. When current is passed through the electrodes, regenerant ions are generated in the first electrode chamber; these ions are pushed into the ion-exchange chamber via an electric field, maintaining the ion exchange chamber in the regenerant form. After counterions exchange from the eluent ion exchange capillary membrane, they are removed from the ion exchange chamber via the electric field into the second electrode chamber. Finally, the counterions are neutralized by the ions generated in the second electrode chamber.

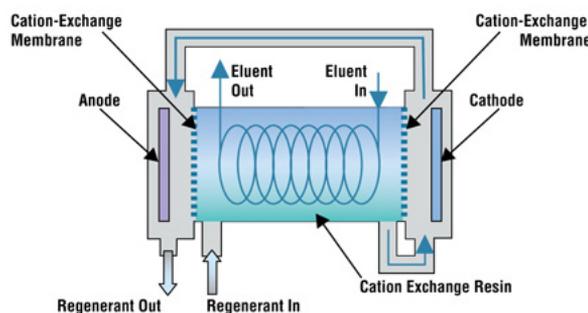


Figure 7. Dionex CES 300 Capillary Electrolytic Suppressor (above) and Dionex CES flow diagram (below).

## Capillary Carbonate Removal Device

In capillary format anion determinations, a carbonate removal device, such as the Dionex CRD 200 or Dionex CRD 180, is an optional device recommended to remove the carbonate peak that interferes with adjoining analyte peaks. For samples with low carbonate content, it is not required, and analysts benefit from sharper peaks due to decreased void volume.

# Capillary Conductivity Detection

The Thermo Scientific Dionex Conductivity Detector handles any IC application, from single column methods with high background signals to determinations of trace contaminants in high purity water. Since all ions are electrically conductive, the conductivity detector is an ideal universal detector for most ion chromatography applications. Suppressed conductivity reduces the background conductivity of the eluent and the strong acid or base forms of the ions are detected by conductivity. The capillary conductivity detector is volume optimized for capillary flow rates and does not require a heat-exchanger.

Features of conductivity detection:

- Optimized for capillary applications
- Minimal cell volume (0.02  $\mu\text{L}$ )
- Microprocessor-controlled digital signal processing detects high and trace concentrations
- Large dynamic range-up to 15,000  $\mu\text{S}/\text{cm}$
- Minimizes noise while maximizing sensitivity



Figure 8. Dionex CD Conductivity Detector

Samples: Beer samples diluted 1:25 with deionized water

- |        |                       |                |
|--------|-----------------------|----------------|
| Peaks: | 1. Quinate            | 8. Carbonate   |
|        | 2. Fluoride           | 9. Sulfate     |
|        | 3. Lactate            | 10. Oxalate    |
|        | 4. Acetate            | 11. Fumarate   |
|        | 5. Pyruvate           | 12. Phosphate  |
|        | 6. Chloride           | 13. Citrate    |
|        | 7. Succinate + Malate | 14. Isocitrate |

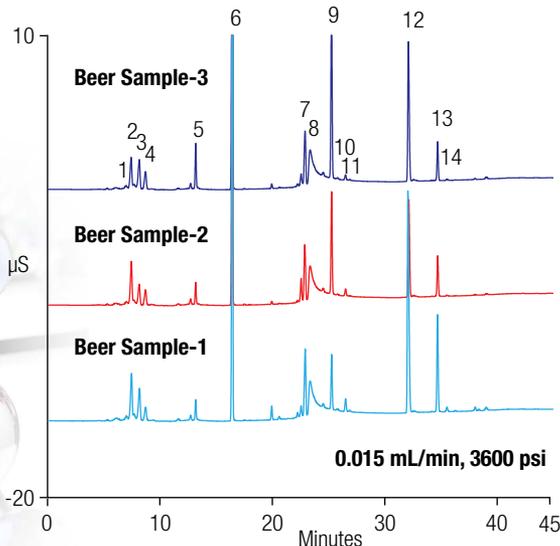


Figure 9. Analysis of anions in fermentation products using a capillary Dionex IonPac AS11-HC-4 $\mu\text{m}$  column.

Peaks:

- |              |           |                |          |
|--------------|-----------|----------------|----------|
| 1. Lithium   | 0.05 mg/L | 8. Putrescine  | 7.5 mg/L |
| 2. Sodium    | 0.20      | 9. Cadaverine  | 4.5      |
| 3. Ammonium  | 0.25      | 10. Histamine  | 6.5      |
| 4. Potassium | 0.50      | 11. Agmatine   | 5.0      |
| 5. Magnesium | 0.25      | 12. Spermine   | 3.0      |
| 6. Calcium   | 0.50      | 13. Spermidine | 1.5      |
| 7. Impurity  | —         |                |          |

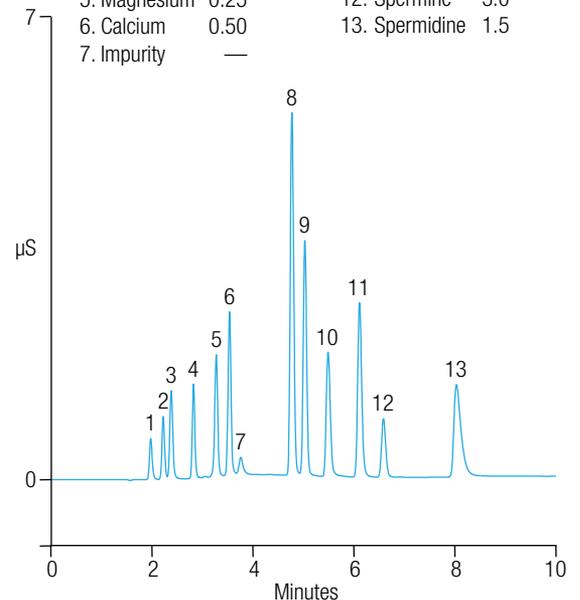


Figure 10. Fast separation of six common cations and biogenic amines using the Dionex IonPac CS19-4 $\mu\text{m}$  capillary column.

## Capillary IC with Electrochemical Detection

Electrochemical detection is used in combination with ion chromatography to detect the presence of compounds that contain an oxidizable or reducible moiety within their structure. Target compounds are separated by on a column and enter the electrochemical detector. The compound is either oxidized or reduced and the flow of electrons is detected as current by the electrochemical detector (ECD). The current linearly correlates to the analyte concentration.

Features of electrochemical detection

- Volume and flow optimized for performance and low backgrounds
- High sensitivity (femtomole detection capability)
- Optional palladium hydrogen reference electrode for superior reproducibility and long lifetime
- Microprocessor-controlled digital signal processing
- DC amperometry, cyclic voltammetry and integrated amperometry including 3-D (current, voltage, retention)



Figure 11. Thermo Scientific Dionex Electrochemical Detector Cell for capillary scale applications.

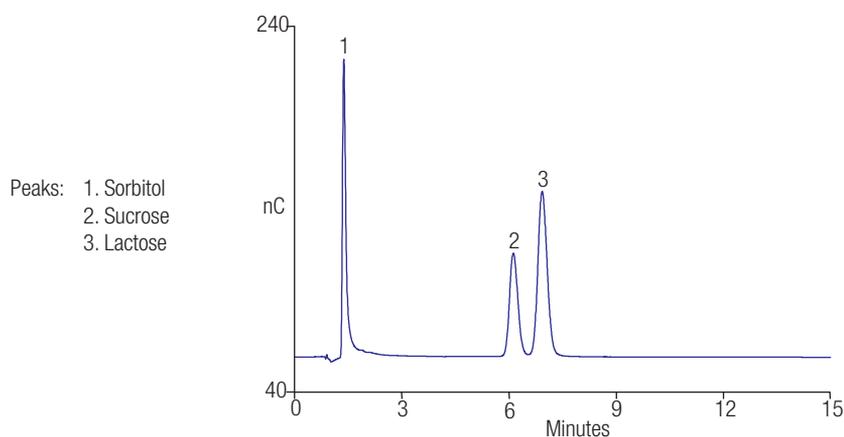


Figure 12. Separation of an alditol and disaccharides using a capillary Dionex CarboPac PA20 column (0.4 x 150 mm).



# Capillary Charge Detection

The Thermo Scientific Dionex QD is the world's first commercially available charge detector for ion chromatography. In combination with conductivity detection, it provides improved peak identification and peak purity analysis. The Dionex QD uses a membrane-based technology and detects ions in proportion to their charge and concentration. Figure 13 illustrates how ions detected by the charge detector yield a signal response that is proportional to the normality of the analyte. It produces up to three times greater signal for triply charged ions, e.g., phosphate, than singly charged ions such as chloride. Ions with the same charge and concentration yield similar response, permitting reliable quantification of known and unknown compounds with a single standard. The Dionex QD is well suited to the analysis of phosphates in environmental samples, organic acids in food and beverages, and amines in chemicals.

Key Features for the Charge Detector:

- Detects all ionizable species
- Stronger relative response for very weakly dissociated and multivalent ions
- Quantification of unknowns possible
- Linear Response for weakly dissociated ions
- Peak purity information
- Complements conductivity detection



Figure 14. Thermo Scientific Dionex QD Charge Detector and the Dionex Conductivity Detector.

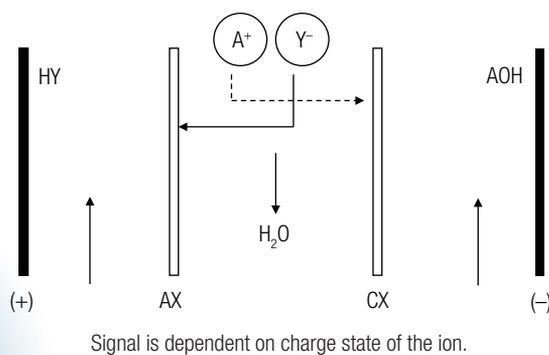


Figure 13. Charge detector operation principle.

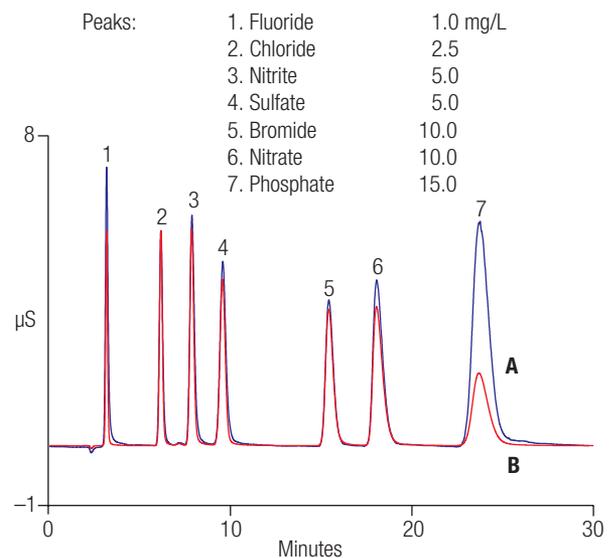


Figure 15. Overlaid chromatograms of a seven anion standard, obtained with suppressed conductivity detection, B, and charge detection, A.

# Capillary Ion Chromatography Column Guide

Thermo Scientific provides a wide range of capillary ion chromatography columns, and our unique column chemistries are designed to solve specific applications. We offer a variety of selectivities and capacities for simple and complex sample matrices to address all your separation needs.

## Dionex IonPac Capillary anion-exchange columns

Column	Market	Application	Analytes
Dionex IonPac AS11-HC-4µm	Food and Beverage	Adulteration, profiling of foods and beverages	Organic acids, inorganic anions
Dionex IonPac AS18-4µm	Environmental	Fast compliance testing of drinking water	Inorganic anions
Dionex IonPac AS19-4µm	Environmental	Compliance monitoring of drinking water	Trace bromate, inorganic anions, oxyhalides
Dionex IonPac AS11-HC	Food and Beverage	Adulteration, profiling of foods and beverages	Organic acids, inorganic anions
Dionex IonPac AS15	Power/ Semiconductor	Trace contamination of high purity water	Inorganic anions, low molecular weight organic acids
Dionex IonPac AS16	Environmental	Compliance testing of drinking water	Trace perchlorate
Dionex IonPac AS18-Fast	Environmental	Fast compliance testing of drinking water and wastewater	Inorganic anions
Dionex IonPac AS18	Environmental	Compliance monitoring of drinking water and wastewater	Inorganic anions
Dionex IonPac AS19	Environmental	Compliance monitoring of drinking water	Trace bromate, inorganic anions, oxyhalides
Dionex IonPac AS20	Environmental	Compliance testing of drinking water	Trace perchlorate
Dionex IonPac AS22	Environmental	Compliance monitoring of drinking water and wastewater	Inorganic anions
Dionex IonPac AS23	Environmental	Compliance monitoring of drinking water and wastewater	Inorganic anions and oxyhalides
Dionex IonPac AS24A	Environmental	Drinking water analysis	Haloacetic acids
Dionex IonPac AS25	Chem/Petrochem	Process monitoring	Sulfur speciation, polyvalent anions
Thermo Scientific™ Dionex™ IonPac AS26	Environmental	Drinking water analysis	Haloacetic acids
Dionex IonSwift MAX-100	Food and Beverage	Adulteration, profiling of foods and beverages	Organic acids, inorganic anions
Dionex IonSwift MAX-200	Environmental	Compliance monitoring of drinking water	Trace bromate, inorganic anions, oxyhalides

## Dionex IonPac Capillary cation-exchange columns

Column	Market	Application	Analytes
Dionex IonPac CS19-4µm	Power/Chemical	Profiling, monitoring in industrial samples	Inorganic cations, small polar amines
Dionex IonPac CS12A-8µm	Environmental	Monitoring of Drinking Water	Inorganic cations plus ammonium
Dionex IonPac CS12A-5µm	Environmental	Monitoring of Drinking Water	Inorganic cations plus ammonium
Dionex IonPac CS16	Power/Chemical	Disparate ratios in industrial samples	Sodium and ammonium
Dionex IonPac CS17	Power/Chemical	Profiling, monitoring in industrial samples	Polyvalent and moderately hydrophobic amines, including diamines
Dionex IonPac CS19	Power/Chemical	Profiling, monitoring in industrial samples	Inorganic cations, small polar amines

## Dionex CarboPac Capillary carbohydrate columns

Column	Market	Application	Analytes
Dionex CarboPac PA20	Food and Beverage	Fast monitoring in food and beverage samples	Mono- and disaccharides
Dionex CarboPac PA10	Food and Beverage	Monitoring in food and beverage samples	Mono- and disaccharides

# Determination of Carbohydrates in Urine by Capillary HPAE-PAD



## Summary

Researchers have studied the presence of certain carbohydrates such as mannitol, rhamnose, xylose, 3-*O*-methylglucose, and lactulose in both urine and serum samples to evaluate disease states in animals and humans. Because carbohydrates are poor chromophores, they are difficult to detect by UV absorption without lengthy and costly derivitization. However, carbohydrates can be determined directly by High Performance Anion-Exchange chromatography with Pulsed Amperometric Detection (HPAE-PAD), a well-established technique that eliminates the need for derivitization, in turn saving time and money (i.e., reagent and disposal costs). This technical note demonstrates a method to determine mono- and disaccharides in synthetic urine on a capillary ion chromatography system.

[Download the full version of Technical Note 137](#)

## Mono- and Disaccharides in Urine

### Equipment

- Dionex ICS-4000 Capillary HPIC System\*
- Dionex IC Cube
- Dionex Electrochemical Detector (ED)
- Dionex Electrochemical Cell, reference electrode with gasket, and working electrode with gasket
- Dionex AS-AP Autosampler
- Thermo Scientific™ Dionex™ Chromeleon™ Chromatography Data system 7 (CDS), version 7.1 with SR2 MUa build or later

Dionex ICS-5000+ can be used for equivalent results

### Reagents and Standards

- 18 MΩ-cm resistivity degassed deionized water
- pH Buffer solutions, pH 7 (Fisher Scientific, P/N SB108-500); pH 10 (Fisher Scientific, P/N SB115-500)

### Conditions

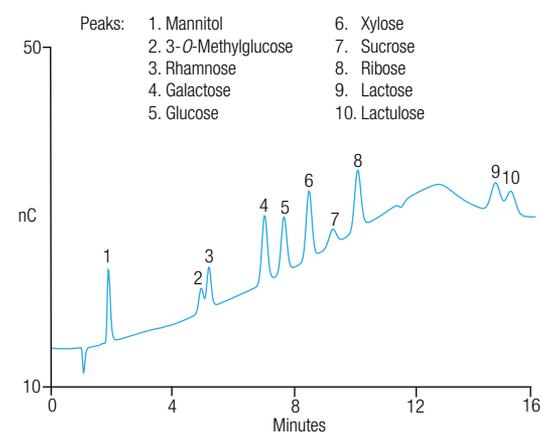
Columns:	Dionex CarboPac PA20 column (0.4 × 150 mm)
Eluent Source:	Thermo Scientific Dionex EGC-KOH Eluent Generator Cartridge (Capillary)
Eluent:	10 mM KOH (–7 to 1 min); 10–30 mM KOH (1 to 9 min); 30–35 mM KOH (9 to 16 min); 35 mM KOH (16 to 21 min); 10 mM KOH (21 to 37 min)
Flow Rate:	0.008 mL/min
Column Temp.:	30 °C
Compartment Temp.:	27 °C
Inj. Volume:	0.4 µL
Detection:	PAD, Gold on PTFE, 0.001" thick gasket, Four-Potential Carbohydrate waveform
Reference Electrode:	pH-Ag/AgCl
Background:	10–20 nC
Noise:	< 10 pC

### Analysis

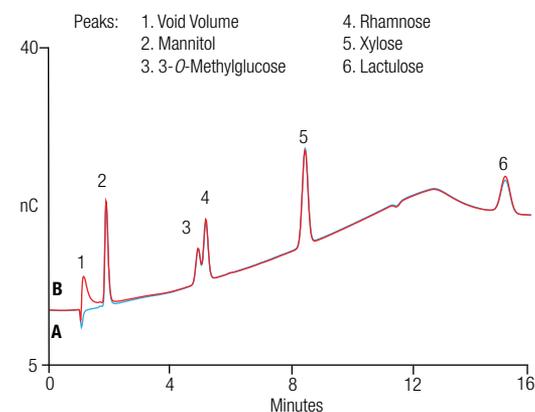
Capillary HPAE-PAD

## Results

See chromatograms below.



Carbohydrates of interest for urine analysis.



Carbohydrates in A) water and B) synthetic urine.

# Quantitative Profiling of Nucleotides Using Capillary IC-MS/MS



## Summary

Nucleoside and nucleotide analogues constitute a major class of antiviral and antitumor agents. These compounds are phosphorylated intracellularly to pharmacologically active nucleoside triphosphates that inhibit DNA synthesis and, subsequently, viral or cell proliferation. Elucidation of the intracellular pharmacology of therapeutic nucleosides and nucleotides is essential for determining drug efficacy and toxicity, and for guiding dosing regimens. Due to high hydrophilicity, nucleotides are commonly analyzed by techniques using ion-exchange chromatography or ion-pair reverse-phase high-performance liquid chromatography. Capillary IC-MS/MS offers unique chromatographic selectivity for polar metabolites and can be used as a complementary technique to reversed-phase LC-MS in metabolomic and clinical research applications.

[Download the full version of Application Note 575](#)

## Quantitation of Mono-, Di-, and Triphosphate Nucleotides

### Equipment

- Dionex ICS-5000 IC Reagent-Free Capillary HPIC system\*
- Dionex ICS-5000 EG Eluent Generator module
- Dionex AS-AP Autosampler
- Thermo Scientific TSQ Quantum Access MAX Triple Quadrupole Mass Spectrometer

\*Dionex ICS-4000 HPIC system and Dionex ICS-5000+ HPIC system can be used for equivalent results

### Chromatographic Conditions

System	Dionex ICS-5000 capillary RFIC	
Column	Dionex IonSwift MAX-100 monolith capillary column (0.25 × 250 mm, P/N 074246) with guard column (0.25 × 50 mm, P/N 074247)	
Eluent source	Thermo Scientific Dionex EGC-KOH cartridge (Capillary) (P/N 072076)	
Eluent	Potassium hydroxide gradient	
	Time (min)	Conc. (mM)
	-5.0	10
	0.1	10
	25.0	75
	29.9	75
	30.0	100
	39.9	100
	40.0	10
Flow rate	0.015 mL/min	
Injection	5 µL	
Temperature	40 °C	
Suppressor	Dionex ACES™ 300 Anion suppressor (P/N 072052) in external water mode	
Suppressor current	20 mA	
Regenerant	Deionized water at 0.030 mL/min	
Desolvation solvent	Acetonitrile at 0.020 mL/min	

### Mass Spectrometric Conditions

Interface	Heated electrospray ionization with HESI II probe and low-flow needle insert (P/N OPTON-53011)
Spray voltage	3500 V
Vaporizer temperature	150 °C
Sheath gas pressure	25 arbitrary units
Auxiliary gas pressure	15 arbitrary units
Capillary temperature	200 °C

### Analysis

Capillary IC-MS/MS

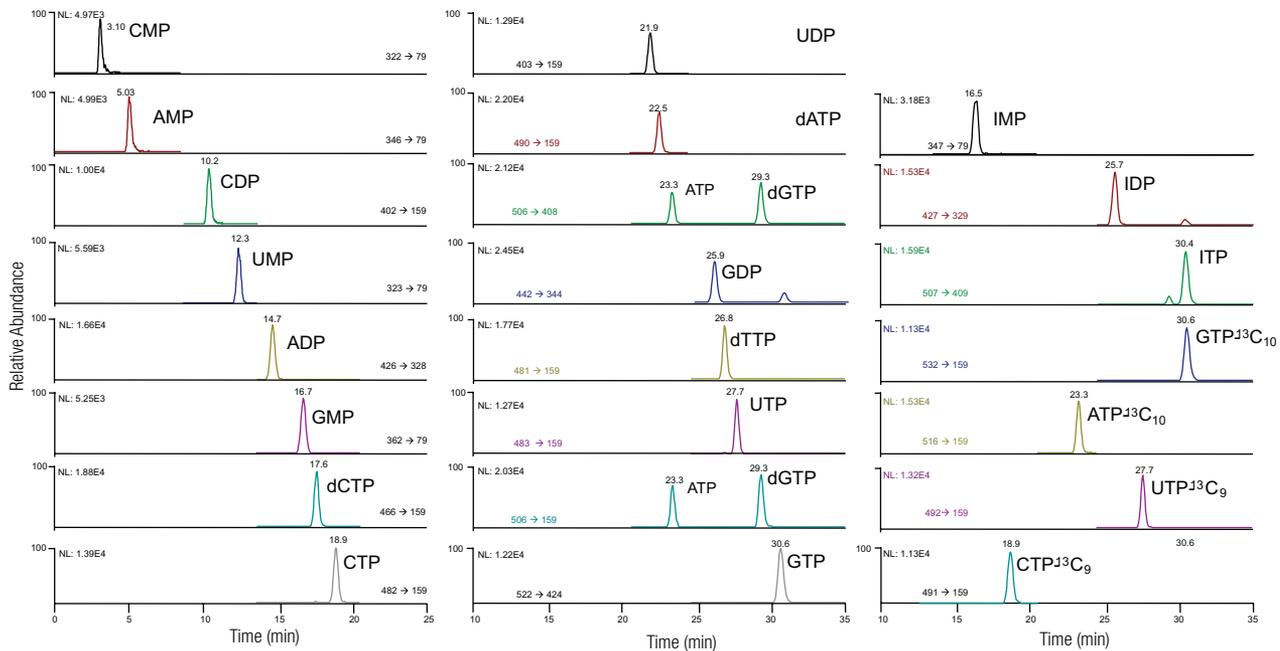
### Results

See table below and chromatogram on next page.

Nucleotide pool content of methylglyoxal-treated and untreated extracts from 293T cells

	Treated (µM)	Untreated (µM)
ADP	13.8	21.2
AMP	> 100	> 100
ATP	0.524	2.53
CDP	5	8.66
CMP	> 40	> 40
CTP	0.351	1.17
dATP	ND	ND
dCTP	ND	ND
dGTP	ND	ND
dTTP	0.085	0.100
GDP	9.05	10.1
GMP	> 100	> 100
GTP	0.374	1.45
IDP	ND	ND
IMP	37.2	23.8
ITP	ND	ND
UDP	7.64	11.6
UMP	> 100	> 100
UTP	0.227	1.52

# Quantitative Profiling of Nucleotides Using Capillary IC-MS/MS



Using a Dionex ICS-5000 RFIC system coupled to a TSQ Quantum Access MAX triple quadrupole MS, a Dionex IonSwift MAX-100 anion-exchange column, and an electrolytically generated hydroxide gradient, 19 native and 2 modified nucleotides were separated and detected within 40 minutes.

# Improved Determination of Trace Perchlorate in Drinking Water Using 2D-IC



## Summary

Perchlorate is identified as an environmental contaminant found in drinking, ground, and surface waters. Research on perchlorate in the environment has received attention because perchlorate poses a human health concern. Perchlorate impairs normal thyroid function by interfering with iodine uptake by the thyroid. Ion chromatography is recognized as an effective tool for the determination of perchlorate in drinking water and other samples. This study demonstrates a 2D-IC system for determining trace concentrations of perchlorate in drinking waters in accordance with EPA Method 314.2 using a capillary format in the second dimension.

[Download the full version of Application Note 1024](#)

## Perchlorate in Drinking Water

### Equipment

- Dionex ICS-5000 Hybrid (Analytical/Capillary) system,\*\* including:
  - DP Dual Pump
  - Dionex EG Eluent Generator
  - DC Detector/Chromatography Compartment
  - Dionex AS-AP Autosampler\* with Sample Syringe, 5.0 mL (P/N 074308) and 8.5 mL buffer line assembly (P/N 075520)
- Dionex Potassium Hydroxide Eluent Generator Cartridge (EGC III KOH, P/N 074532) and Capillary Cartridge (EGC KOH, P/N 072076)
- Thermo Scientific Dionex CR-ATC Continuously Regenerated Anion Trap Column (P/N 060477) and Capillary Trap Column (P/N 072078)
- Dionex CRD 200 Carbonate Removal Device, 2 mm (P/N 062986) and Capillary Dionex CRD 200 Carbonate Removal Device, (P/N 072054)
- Dionex IonSwift MAC-200 Monolith Anion Concentrator Column (P/N 075461)
- Vial Kit, Polystyrene with Caps and Blue Septa, 10 mL (P/N 074228)
- Dionex IC Cube Cartridge with six-port valve (P/N 078841)
- Corning™ Syringe Filter, surfactant-free cellulose acetate (SFCA), 0.2 µm pore, 26 mm (Fisher Scientific (P/N 09-754-13)
- Disposable syringe, 20 mL (24 mL) Luer Lock, Sterile (Fisher Scientific P/N 14-817-33)
- Sterile sample container, 125 mL, I-Chem™ Sterile Nalgene™ Bottles (Fisher Scientific P/N N411-0125)
- PEEK tubing, 38 cm (15 in.) piece of 0.025 mm (0.001 in.) i.d. (P/N 074582) for conditioning a new capillary Dionex EGC cartridge

\*A Dionex AS or AS-DV Autosampler can also be used for sample delivery

\*\*Dionex ICS-5000\* HPIC System can be used for equivalent results

### Reagents and Standards

- Deionized water (DI), Type I reagent grade, 18 MΩ-cm resistance or better
- Sodium perchlorate, 98%, extra pure (Fisher Scientific P/N AC34218)
- Sodium chloride, crystalline, 99.0% (Fisher Scientific P/N S671)
- Sodium sulfate, anhydrous (Fisher Scientific P/N S429)
- Sodium bicarbonate, certified ACS (Fisher Scientific P/N S233)

### First Dimension Conditions

Columns:	Dionex IonPac AG20 Guard column, 2 × 50 mm (P/N 063066)
	Dionex IonPac AS20 Analytical column, 2 × 250 mm (P/N 063065)
Eluent Source:	Dionex EGC III KOH Eluent Generation Cartridge with Dionex CR-ATC Continuously Regenerated Anion Trap Column
Eluent:	35 mM KOH 0–30 min, step to 60 mM at 30.1 min, 60 mM 30.1–40 min, step to 35 mM at 40.1 min, 35 mM 40.1–45 min
Flow Rate:	0.25 mL/min
Injection Volume:	500 µL
Temperature:	15 °C (upper compartment) 30 °C (lower compartment)
Detection:	Suppressed conductivity, Thermo Scientific™ Dionex™ ASRS™ 300 Anion Self-Regenerating Suppressor, 2 mm, 38 mA, external water mode
System	
Backpressure:	~2185 psi
Background	
Conductance:	~0.500 µS
Noise:	~0.3 nS/min peak-to-peak
Run Time:	45 min

## Second Dimension Conditions

Columns: Dionex IonPac AG16 Capillary Guard column, 0.4 × 50 mm (P/N 082316)

Dionex IonPac AS16 Capillary Analytical column, 0.4 × 250 mm (P/N 082315)

Eluent Source: Dionex EGC-KOH Cartridge (Capillary) with Dionex CR-ATC Continuously Regenerated Anion Trap Column (Capillary)

Eluent: 65 mM KOH

Flow Rate: 0.01 mL/min

Injection Volume: 1 mL (on the concentrator column from first dimension)

Temperature: 15 °C (upper compartment)  
30 °C (Dionex IC Cube Cartridge)

Detection: Suppressed conductivity, Dionex ACES 300 suppressor (P/N 072052), 12 mA, external water mode

System Backpressure: ~1230 psi

Background Conductance: ~0.400 µS

Noise: ~0.5 nS/min peak-to-peak

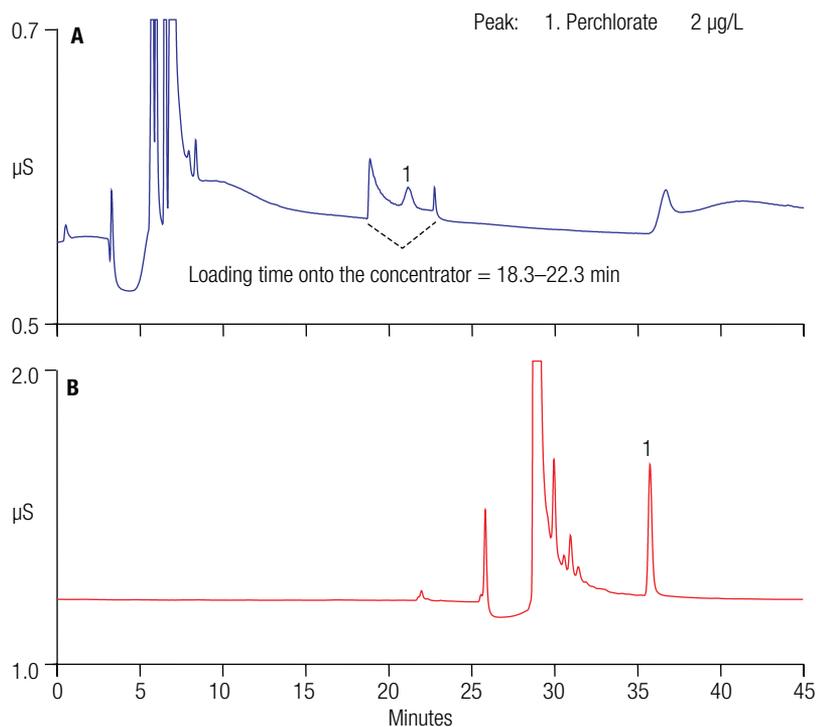
Run Time: 45 min

## Analysis

2D-IC

## Results

See chromatogram below.



Chromatogram of a 2 µg/L perchlorate standard in reagent water in (A) first dimension and (B) second dimension.

# Determination of Nitrite and Nitrate in Wastewater Using Capillary IC with UV Detection



## Summary

Ion chromatography with suppressed conductivity detection is an effective technique to simultaneously determine common inorganic anions in environmental water and drinking water. However in some samples, such as mineral water, wastewater, and brine, accurate quantification of some anions present at low concentrations can be challenging due to the high ionic strength of the sample. Ion chromatography with UV detection provides an alternate approach for determining nitrite and nitrate without compromising sensitivity. By combining suppressed conductivity with UV detection, the suppressor reduces the background noise. The Dionex Capillary RFIC system delivers fast turnaround by reducing eluent preparation, system startup, and equilibration times. This method is a solution for nitrite analysis when high concentrations of chloride can mask the presence of nitrite.

[Download the full version of Application Update 185](#)

## Nitrite and Nitrate in Wastewater

### Equipment

- Dionex ICS-5000 Capillary IC system\* including:
  - DP Dual Pump module (Capillary)
  - Dionex EG Eluent Generator module with Dionex EGC (Capillary) and Dionex CR-TC (Capillary)
  - DC Detector/Chromatography Compartment with Dionex IC Cube module and Capillary CD Conductivity Detector
  - Dionex AS-AP Autosampler
  - Dionex ICS-Series VWD Variable Wavelength
- Detector with PEEK capillary cell (P/N 076072)
- Thermo Scientific™ Dionex™ Chromeleon™ Chromatography Data System (CDS) software

\*Dionex ICS-4000 HPIC System and Dionex ICS-5000+ HPIC System can be used for equivalent results

### Reagents and Standards

- Nitrite, 1000 mg/L (Fisher Scientific P/N AS-NO29-27)
- Nitrate, 1000 mg/L (Fisher Scientific P/N AS-NO3N9-2y)

### Conditions

Column:	Dionex IonPac AS18-Fast column, 0.4 × 150 mm
Eluent:	33 mM KOH
Flow Rate:	0.015 mL/min/min
Inj. Volume:	0.4 µL
Detection:	A: Suppressed conductivity, Thermo Scientific Dionex ACES 300 suppressor, recycle mode B: UV, 210 nm, capillary

### Analysis

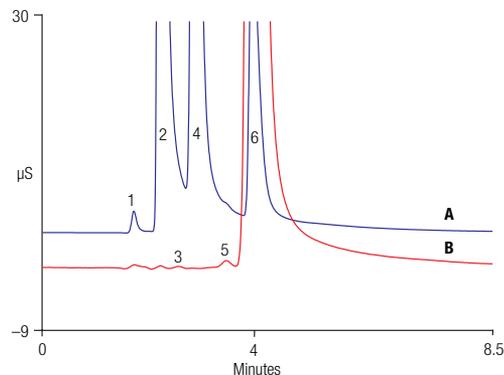
Capillary Ion Chromatography

### Results

See chromatogram below.

Column:	Dionex IonPac AS18-Fast column, 0.4 mm × 150 mm
Eluent Source:	EGC-KOH, cartridge (Capillary)
Eluent:	33 mM KOH
Flow Rate:	15 µL/min
Inj. Volume:	0.4 µL
Column Temp.:	30 °C
Detection:	A. Suppressed conductivity, capillary B. UV, 210 nm, capillary
Sample Prep:	Filtered prior to analysis

Peaks:	A	B	mg/L
1. Fluoride	—	—	—
2. Chloride	—	—	—
3. Nitrite	—	0.030	—
4. Sulfate	—	—	—
5. Bromide	—	—	—
6. Nitrate	48.7	48.7	—



Separation of inorganic anions in a municipal wastewater sample spiked with 0.030 mg/L nitrite.

# Determination of Inorganic Anions in Acid Rain Using a Dedicated High-Pressure Capillary Ion Chromatograph



## Summary

Acid rain is closely monitored by many countries due to its negative effects on plants, aquatic animals, infrastructures, and human health. The cause of acid rain is related to the reaction of water in the atmosphere with sulfur dioxide (SO<sub>2</sub>) and nitrous oxides (NO<sub>x</sub>) released from natural phenomena, such as volcanic eruption and lightning strikes. Some human activities, for example, coal power plants and motor vehicles, contribute to the level of SO<sub>2</sub> and NO<sub>x</sub> in the air as well. Air and rain monitoring has become an important task worldwide for investigating the effects of pollutants on the global ecology and assessing the advancement of pollution control. This method demonstrates rapid analysis of determining inorganic anions in rainwater with a high-pressure capillary IC and 4 μm particle-size columns.

[Download the full version of Technical Note 124](#)

## Inorganic Anions in Acid Rain

### Equipment

- Dionex ICS-4000 HPIC system\*
- Dionex AS-AP Autosampler
- Chromeleon Chromatography Data System 7.1 with SR2 MUa build or later

\*Dionex ICS-5000+ HPIC System can be used for equivalent results

### Reagents and Standards

- 18 MΩ-cm degassed deionized water
- Fluoride standard 1000 mg/L (Thermo Scientific Dionex P/N 037158)
- Chloride standard 1000 mg/L (Thermo Scientific Dionex P/N 037159)
- Sulfate standard 1000 mg/L (Thermo Scientific Dionex P/N 037160)
- Nitrate standard 1000 mg/L (Thermo Scientific Dionex P/N 056497)
- Bromide standard 1000 mg/L (Ultra Scientific P/N ICC-001)
- Nitrite standard 1000 mg/L (Ultra Scientific P/N ICC-007)
- Phosphate standard 1000 mg/L (Ultra Scientific P/N ICC-005)

### Conditions

Columns:	Dionex IonPac AG18-4μm column, 0.4 × 50 mm Dionex IonPac AS18-4μm column, 0.4 × 150 mm
Eluent Source:	Dionex EGC-KOH Cartridge (Capillary)
Eluent:	23 mM KOH
Flow Rate:	A: 0.025 mL/min for sample B: 0.010, 0.020, and 0.025 mL/min for standards
Column Temp.:	30 °C
Compartment Temp.:	15 °C
Inj. Volume:	0.4 μL (full loop injection mode)
Detection:	Suppressed conductivity, Dionex ACES 300 suppressor, recycle mode, 8 mA at 0.010 mL/min; 13 mA at 0.025 mL/min
Background Conductance:	< 1 μS
Noise:	< 1 nS
System Backpressure:	~1600 psi (0.010 mL/min); ~2900 psi (0.020 mL/min); ~3500 psi (0.025 mL/min)

### Analysis

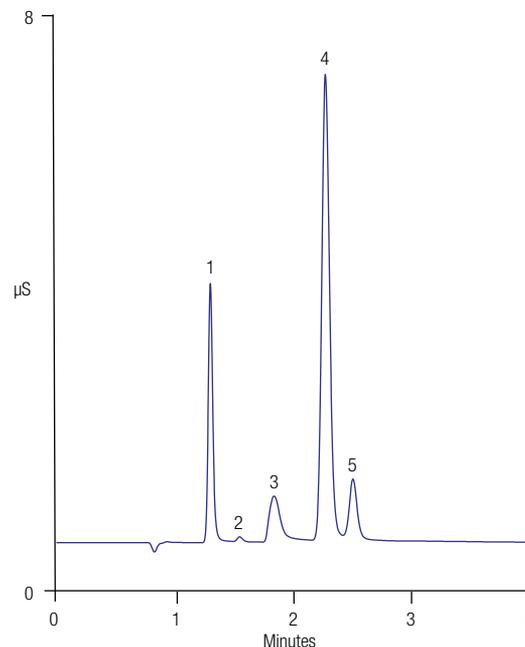
Capillary Ion Chromatography

### Results

See chromatogram below.

Column :	Dionex IonPac AG18 column, AS18-4μm column, capillary		
Eluent Source:	Dionex EGC-KOH Cartridge (Capillary)		
Eluent:	23 mM KOH		
Col. Temp.:	30 °C		
Inj. Volume:	0.4 μL		
Flow Rate:	0.025 mL/min		
Detection:	Suppressed Conductivity, Dionex ACES 300 suppressor		
Sample:	Rain sample		

Peaks:			mg/L
1. Chloride	1.0		
2. Nitrite	0.03		
3. Carbonate	—		
4. Sulfate	5.2		
5. Nitrate	0.78		



Fast determination of inorganic anions in a rain water sample.

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



## Summary

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. 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, thereby saving money and labor.

[Download the full version of Technical Note 118](#)

## Anions and Organic Acids in Carbonated Beverages

### Equipment

- Dionex ICS-5000+ IC Reagent-Free Capillary HPIC system\*
- Dionex ICS-5000+ EG Eluent Generator module
- Dionex ICS-5000+ DC Detector/Chromatography module with Dionex IC Cube cartridge and high-pressure degas cartridge
- Dionex AS-AP Autosampler
- Chromeleon Chromatography Data system

\*Dionex ICS-4000 HPIC System can be used for equivalent results

### Reagents and Standards

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

### Conditions

Columns:	Dionex IonSwift MAX 200 column, 0.25 $\times$ 250 mm
Eluent Source:	Dionex EGC KOH Eluent Generator Capillary Cartridge with 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: 0.010; B: 0.020; C: 0.025 mL/min/min
IC Cube Temp.: *	30 $^{\circ}$ C
Compartment Temp.:	15 $^{\circ}$ C
Inj. Volume:	0.4 $\mu$ L
Detection:	Suppressed conductivity, Dionex ACES 300 suppressor, Dionex CRD 200 Carbonate Removal Device (Capillary), recycle mode, A: 8 mA; B: 15 mA; C: 18 mA

### Background

Conductance: 0.5–0.8  $\mu$ S 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.

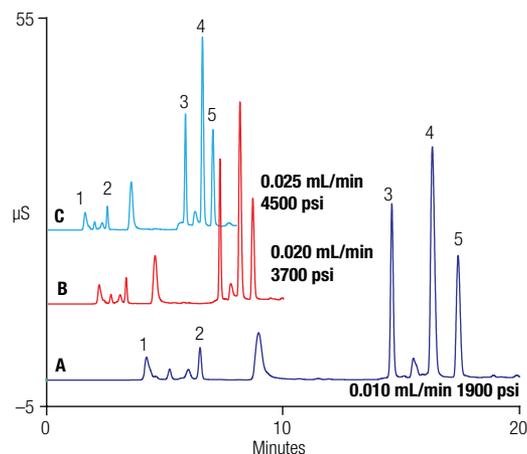
### Analysis

Capillary Ion Chromatography

### Results

See chromatogram below.

Column:	Dionex IonSwift MAX-200G, MAX-200 columns, 0.25 mm $\times$ 250 mm												
Eluent Source:	Dionex EGC-KOH cartridge (Capillary)												
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 $\mu$ L												
Column Temp.:	30 $^{\circ}$ C												
Detection:	Suppressed Conductivity, Dionex ACES 300 suppressor, Dionex CRD 200 (Capillary) Carbonate Removal Device												
Sample Prep.:	Degassed, diluted 10-fold, filtered, 0.45 $\mu$ m												
Peaks:	<table border="1"> <thead> <tr> <th></th> <th>Total</th> </tr> </thead> <tbody> <tr> <td>1. Chloride</td> <td>26.0 mg/L</td> </tr> <tr> <td>2. Nitrate</td> <td>7.3</td> </tr> <tr> <td>3. Sulfate</td> <td>50.3</td> </tr> <tr> <td>4. Phosphate</td> <td>226.0</td> </tr> <tr> <td>5. Citrate</td> <td>48.1</td> </tr> </tbody> </table>		Total	1. Chloride	26.0 mg/L	2. Nitrate	7.3	3. Sulfate	50.3	4. Phosphate	226.0	5. Citrate	48.1
	Total												
1. Chloride	26.0 mg/L												
2. Nitrate	7.3												
3. Sulfate	50.3												
4. Phosphate	226.0												
5. Citrate	48.1												



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

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



## Summary

Determinations of organic acid profiles in fruit juices are used in the beverage industry to characterize flavor, identify spoilage and potential sources of adulteration, and to meet labeling requirements for food products. In this study, inorganic anions and organic acids from a diluted orange juice sample are separated by anion exchange chromatography on a capillary size Dionex IonSwift MAX-100 monolith IC column and detected by suppressed conductivity detection, using the Dionex ACES Anion Capillary Electrolytic Suppressor specifically optimized for capillary IC. 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.

[Download the full version of Technical Note 119](#)

## Organic Acids in Fruit Juice

### Equipment

- Dionex ICS-5000+ IC Reagent-Free Capillary IC system\*
- Dionex ICS-5000+ EG Eluent Generator module
- Dionex ICS-5000+ DC Detector/Chromatography module with Dionex IC Cube cartridge and high-pressure degas cartridge
- Dionex AS-AP Autosampler
- Chromeleon Chromatography Data System, version 6.8 and 7.1

\*Dionex ICS-4000 HPIC system can be used for equivalent results

### Reagents and Standards

- 18 M $\Omega$ -cm degassed deionized water
- Dionex Combined Seven Anion II Standard (Dionex P/N 057590)

### Conditions

Columns:	Dionex IonSwift MAX 100 guard and separation column set, 0.25 × 250 mm
Eluent Source:	Dionex EGC KOH cartridge (Capillary) 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 suppressor, Dionex CRD 200 Carbonate Removal Device (Capillary), recycle mode A: 8 mA B: 18 mA
Background Conductance:	< 1.0 $\mu$ S
Noise:	< 1.0 nS
System backpressure:	A: ~ 2100 psi B: < 4200 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.

### Analysis

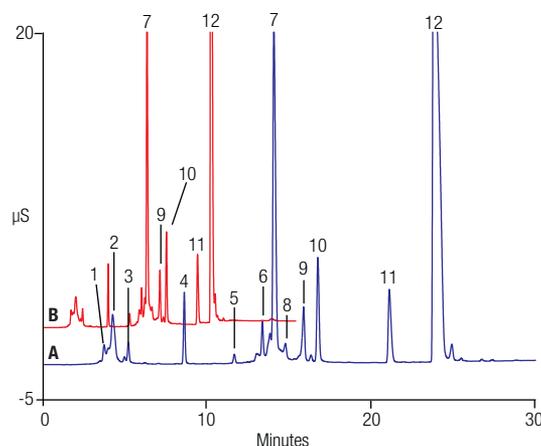
Capillary Ion Chromatography

### Results

See chromatogram below.

Column:	Dionex IonSwift MAX-100 guard column Dionex MAX-100 column, 0.25 mm		
Eluent Source:	Dionex EGC-KOH cartridge (Capillary), 0.25 mm		
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: 0.012 mL/min; B: 0.024 mL/min		
Inj. Volume:	0.4 $\mu$ L		
Detection:	Suppressed conductivity, Dionex ACES 300 suppressor, recycle mode		
Sample Prep.:	1:40 dilution, filtered, 0.45 $\mu$ m		
Peaks:	1. Quinate 2.0 mg/L	8. Maleate 0.1 mg/L	
	2. Lactate 3.2	9. Sulfate 2.0	
	3. Formate 0.7	10. Oxalate 4.6	
	4. Chloride 1.0	11. Phosphate 7.5	
	5. Nitrate —	12. Citrate 125	
	6. Glutarate —		
	7. Malate 25		

A: 0.012 mL/min 2100 psi  
B: 0.024 mL/min 4200 psi



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

# Determinations of Monosaccharides and Disaccharides in Beverages by Capillary HPAE-PAD



## Summary

Mono- and disaccharide sugar determinations are often used in the food and beverage industry to ensure the quality of a formulated product, to maintain or select for desired sweetness, and to characterize and confirm the source of the carbohydrates. Carbohydrates have poor chromophores and are therefore problematic to detect by UV absorption without lengthy and costly derivitization. However, carbohydrates can be determined directly by High Performance Anion-Exchange chromatography and Pulsed Amperometric Detection (HPAE-PAD), a well-established method that eliminates the need for derivitization, saving time and money including reagent costs. This application demonstrates mono- and disaccharides determinations in two-fold to 10,000-fold diluted beverage samples by HPAE-PAD at capillary flow rates on the Dionex ICS-4000 HPIC Integrated capillary system.

[Download the full version of Technical Note 135](#)

## Mono- and Disaccharides in Beverages

### Equipment

- Dionex ICS-4000 Capillary HPIC System\*
- Dionex IC Cube
- Dionex Electrochemical Detector (ED)
- Thermo Scientific Dionex Electrochemical Cell, reference electrode with gasket, and working electrode with gasket
- Dionex AS-AP Autosampler
- Chromeleon Chromatography Data System, version 7.1 with SR2 MUa build or later

\*Dionex ICS-5000+ HPIC system can be used for equivalent results

### Reagents and Standards

- 18 M $\Omega$ -cm degassed deionized water
- ACS Grade reagents, Fisher Scientific
- Thermo Scientific Dionex MonoStandard, Mixture of Six, 100 nmol each (P/N 043162)
- pH Buffer solutions, (pH 7 / pH 10) (Fisher Scientific, P/N SB108-500 / SB115-500)

### Conditions

Columns:	Dionex CarboPac PA20 column set, 0.4 × 150 mm
Eluent Source:	Dionex EGC-KOH Eluent Generator Cartridge (Capillary)
Eluent*:	10 mM KOH
Flow Rate:	0.008 mL/min
Column Temp.:	30 °C
Compartment Temp.:	27 °C
Inj. Volume:	0.4 $\mu$ L
Detection:	PAD, Gold on PTFE, 0.001" or 0.015" gasket, Four-Potential Carbohydrate waveform
Reference Electrode:	pH-Ag/AgCl
Background:	10–20 nC
Noise:	< 10 pC

\*Column wash/10 samples: 5 min at 100 mM KOH, 7 min equilibration at 10 mM KOH.

### Analysis

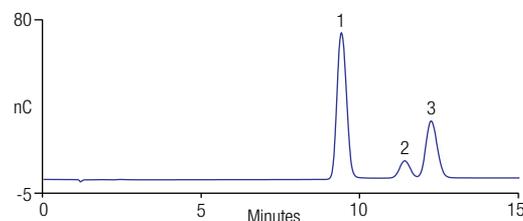
Capillary HPAE-PAD

### Results

See chromatograms below.

Column:	Dionex CarboPac PA20 column, 0.4 × 150 mm		
Eluent Source:	Dionex EGC-KOH Cartridge (Capillary)		
Eluent:	10 mM KOH (15 min)		
Flow Rate:	0.008 mL/min		
Inj. Volume:	0.4 $\mu$ L		
Column Temp.:	30 °C		
Detection:	PAD, Au disposable, 0.001" gasket, 4-Potential Carbohydrate waveform		
Ref. Electrode:	Ag/AgCl		
Sample Prep.:	10,000-fold dilution		

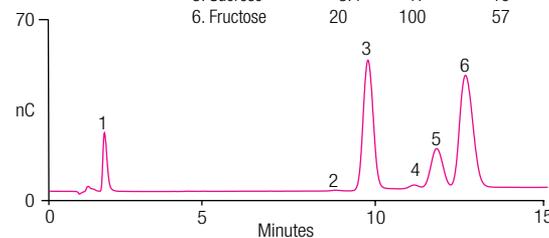
Peaks:	mg/L	Total	% Ratio
1. Glucose	4.6	46 g/L	39
2. Sucrose	1.3	13	11
3. Fructose	5.9	59	50



Glucose, sucrose, and fructose in tea beverage.

Column:	Dionex CarboPac PA20 column set, 0.4 × 150 mm		
Eluent Source:	Dionex EGC-KOH Cartridge (Capillary)		
Eluent:	10 mM KOH (15 min)		
Flow Rate:	0.008 mL/min		
Inj. Volume:	0.4 $\mu$ L		
Column Temp.:	30 °C		
Detection:	PAD, Au disposable, 0.001" gasket, 4-Potential Carbohydrate waveform		
Ref. Electrode:	Ag/AgCl		
Sample Prep.:	10,000-fold dilution		

Peaks:	mg/L	Total	% Ratio
1. Void Volume			
2. Galactose	< 0.02	— g/L	—
3. Glucose	11	55	32
4. Mannose	0.4	2	1
5. Sucrose	3.4	17	10
6. Fructose	20	100	57



Diluted apple cider with native sugar only.

# Quantitative Determination of Bisphosphonate Pharmaceuticals and Excipients by Capillary IC-MS



## Summary

Bisphosphonates are a group of compounds that are used as active pharmaceutical ingredients (APIs) to treat bone disorders including osteoporosis, Paget's disease, and hypercalcemia. Typical methods for bisphosphonates analysis include liquid chromatography (LC) with derivatization and/or ion pairing, ion chromatography (IC), capillary electrophoresis (CE), and gas chromatography (GC) with derivatization. This application note discusses a quantitative approach for the direct analysis of bisphosphonates and excipients in pharmaceuticals using capillary IC with suppressed conductivity and mass spectrometric detection. This method is used to provide chromatographic retention and resolution for target analytes, and the elimination of derivatization steps simplifies the workflow and improves method throughput.

[Download the full version of Application Note 1001](#)

## Bisphosphonate and Common Excipients in Pharmaceuticals

### Equipment

- Dionex ICS-5000 Capillary HPIC System\* with Eluent Generation
- Thermo Scientific MSQ Plus Mass Spectrometer (single quadrupole)
- Dionex AXP-MS Auxiliary pump (x2)
- Chromeleon Chromatography Data System, version 6.8 SR11
- Thermo Scientific™ Xcalibur 2.0.7 with MSQ™ 2.0 SP1

\*Dionex ICS-5000+ and Dionex ICS-4000 can be used for equivalent results

### Reagents and Chemicals\*

- Etidronate disodium hydrate (PN P5248)
- Clodronate disodium (PN D4434)
- Tiludronate disodium hydrate (PN T4580)
- Benzoic acid sodium salt (PN B3375)
- *p*-Hydroxybenzoic acid (PN H5376)
- Citric acid (PN 27788)
- Isotope labeled internal standard citric acid-d4 (C/D/N Isotopes, Inc., PN D-3745)
- Deionized (DI) water with 18.2 MΩ-cm resistivity
- Acetonitrile (LC/MS grade, Fisher Scientific or equivalent)

All chemical standard chemicals were purchased from Sigma-Aldrich unless noted.

### Chromatographic Conditions

System:	Dionex ICS-5000 capillary IC system with eluent generation	
Column:	Dionex IonPac AS18-Fast Capillary Column (0.4 × 150 mm, PN 072062)	
	Dionex IonPac AG18-Fast Capillary Guard Column (0.4 × 35 mm, PN 072063)	
Eluent:	Hydroxide gradient	
	Time (min)	Concentration (mM)
	-4.0	40
	0.0	40
	5.0	50
	8.0	100
	13.9	100
	14.0	40

Eluent Source: Dionex EGC-KOH (Capillary) Cartridge (PN 072076)

Flow Rate: 0.020 mL/min

Injection: 2 µL

Temperature: 40 °C

Detection: 1) Suppressed conductivity with Dionex ACES 300 suppressor (external water mode, 30 µL/min DI water delivered by AXP-MS pump)

2) MSQ Plus single quadrupole mass spectrometer

### Mass Spectrometric Conditions

System:	MSQ Plus mass spectrometer, single quadrupole
Interface:	Capillary low-flow electrospray ionization (ESI) negative polarity
Probe:	MSQ Plus ESI probe with low-flow option (PN 078996)
Probe Temperature:	300 °C
Needle Voltage:	3500 V
Desolvation Solvent:	20 µL/min acetonitrile delivered by a Dionex AXP-MS pump
Nebulizer Gas:	Nitrogen at 65 psi
Acquisition:	Selected ion monitoring (SIM) with cone set at 55 V for each SIM with 0.3 amu span

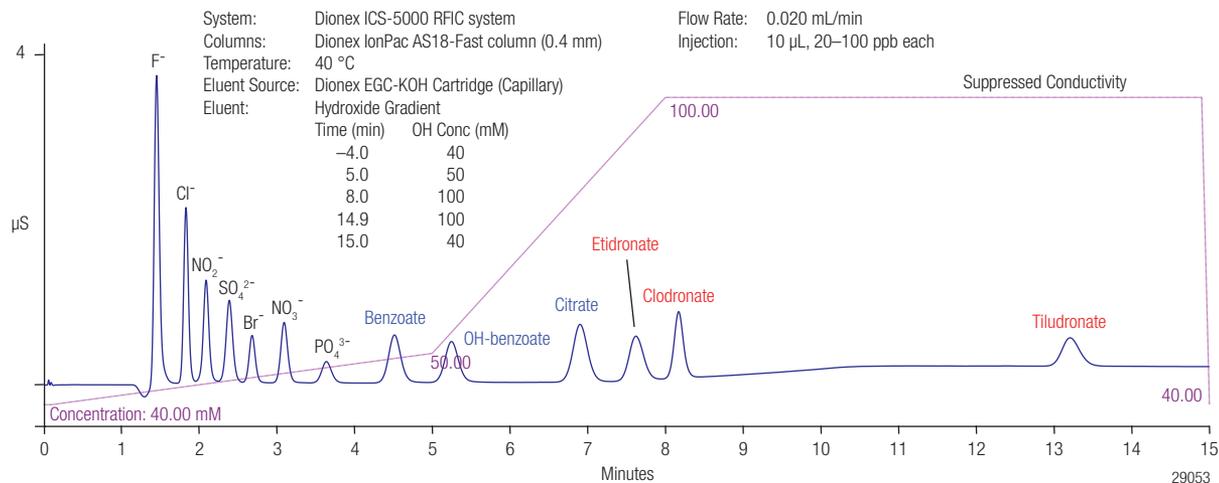
# Quantitative Determination of Bisphosphonate Pharmaceuticals and Excipients by Capillary IC-MS

## Analysis

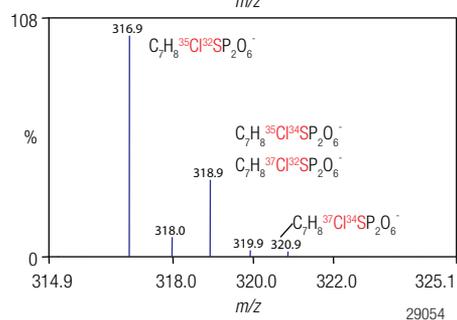
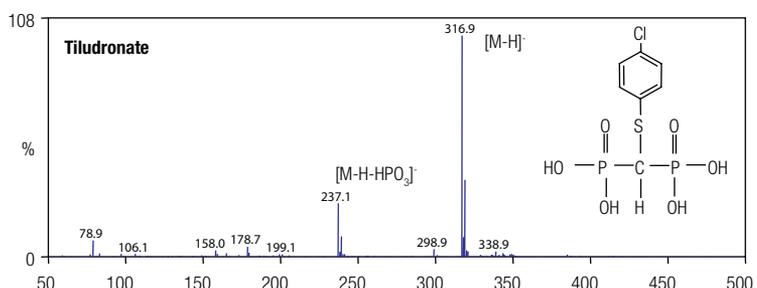
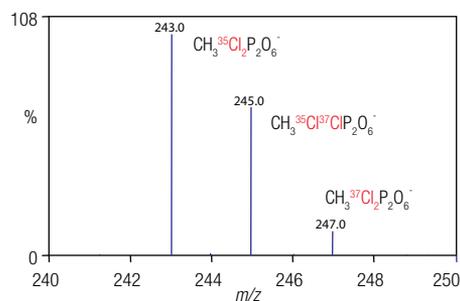
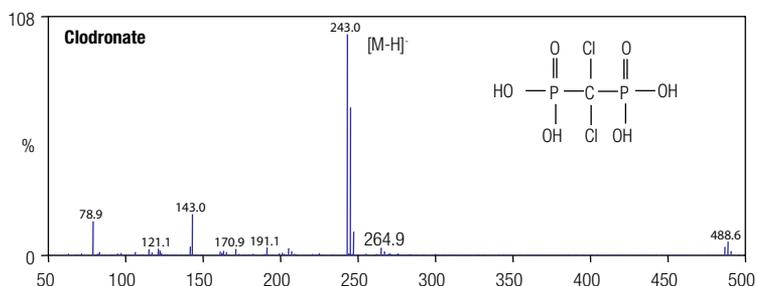
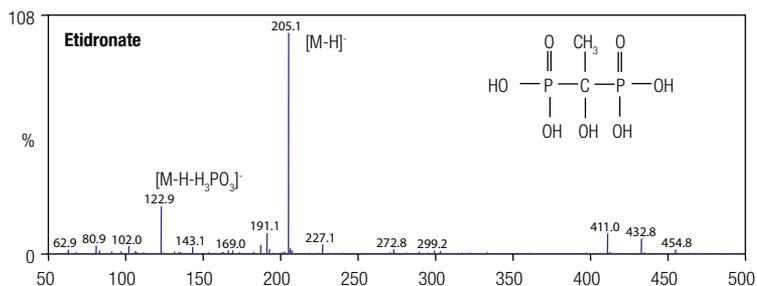
Capillary IC-MS

## Results

See chromatogram and figures below.



Total resolution of bisphosphonates, excipients and anions.



FMS Spectra of three bisphosphonate pharmaceuticals.

# Implementation of a Walk-Up High-Pressure Capillary Ion Chromatograph for the Fast Separation of Pharmaceutical Relevant Inorganic Anions and Cations



## Summary

Ion chromatography (IC) with suppressed conductivity detection is a well-established technique for the determination of inorganic and organic ions in pharmaceuticals. This work describes the development of a walk-up IC system using high-pressure to provide ultrafast separations of inorganic anions and cations relevant to the pharmaceutical industry. Data will be presented on the identification, quantification, and control of inorganic impurities that are important during drug development, and the benefits an Always Ready system brings to IC analysis. Additionally, ion exchange with suppressed conductivity detection provides a highly sensitive and selective detection mode for the analysis of counterions in pharmaceutical formulations.

[Download the full version of Application Note 2967](#)

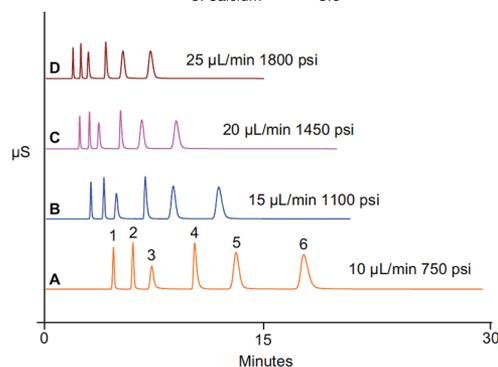
## Anions and Cations in Pharmaceuticals

### Equipment

- Dionex ICS-5000 Reagent-Free Capillary IC system\* consisting of:
  - DP Dual isocratic capillary pump
  - DC Detector and Chromatography Module
  - Dionex IC Cube capillary module compartment
  - CD Capillary Conductivity Detector for Anions and Cations
  - EG Eluent Generator
  - Dionex AS-AP Autosampler with diverter valve
- Chromeleon Chromatography Data System

\*Dionex ICS-5000+ HPIC system and Dionex ICS-4000 HPIC system can be used for equivalent results

Column:	Dionex IonPac CS16 column, Capillary 0.5 × 250 mm																		
Eluent Source:	Dionex EGC-MSA Cartridge (Capillary)																		
Eluent:	30 mM MSA																		
Peaks:	<table border="1"> <tr><td>1. Lithium</td><td>0.5</td><td>mg/L</td></tr> <tr><td>2. Sodium</td><td>2.0</td><td></td></tr> <tr><td>3. Ammonium</td><td>2.5</td><td></td></tr> <tr><td>4. Potassium</td><td>5.0</td><td></td></tr> <tr><td>5. Magnesium</td><td>2.5</td><td></td></tr> <tr><td>6. Calcium</td><td>5.0</td><td></td></tr> </table>	1. Lithium	0.5	mg/L	2. Sodium	2.0		3. Ammonium	2.5		4. Potassium	5.0		5. Magnesium	2.5		6. Calcium	5.0	
1. Lithium	0.5	mg/L																	
2. Sodium	2.0																		
3. Ammonium	2.5																		
4. Potassium	5.0																		
5. Magnesium	2.5																		
6. Calcium	5.0																		



Fast cation determinations by high-pressure capillary IC.

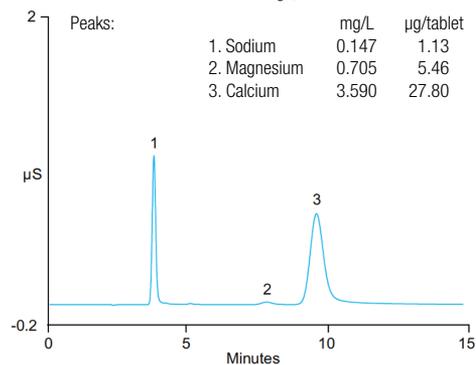
### Analysis

Capillary Ion Chromatography

### Results

See chromatograms below.

Column:	Dionex IonPac CS12A column, 0.4 × 250 mm
Eluent Source:	Dionex EGC-MSA Cartridge (Capillary)
Gradient:	20 mM MSA
Flow Rate:	0.010 mL/min
Inj. Volume:	0.4 µL
Column Temp.:	30 °C
Detection:	Dionex CCES 300 suppressor
Sample:	1.29 mg/mL of 10 mg tablet in water
Sample Prep:	Filtered, Thermo Scientific™ Dionex™ OnGuard™ RP Cartridge, 1:10 dilution



Counterion determinations in a atorvastatin calcium tablet by capillary IC.

# Fast Separations of Counter Ion in an Allergy Drug Tablet Using High-Pressure Capillary IC



## Summary

Many drugs are manufactured in a salt form to provide solubility, bioavailability, and stability. Counter ion determinations are important to confirm the correct stoichiometry and formula weight of the drug but also to test for drug impurities. In this study, inorganic anions from an allergy treatment pharmaceutical tablet dissolved in water are separated by anion-exchange chromatography on a capillary size monolith column. This application demonstrates the advantages of high-pressure capillary IC using a high efficiency capillary size anion-exchange monolith column to provide high sample throughput by simply increasing the flow rate on a high-pressure capable capillary Dionex HPIC system.

[Download the full version of Technical Note 120](#)

## Separation and Quantification of Counter Ions in Pharmaceuticals

### Equipment

- Dionex ICS-5000+ HPIC Reagent-Free capillary IC\* system including:
  - Dionex ICS-5000+ SP Single Pump or DP Dual Pump module
  - Dionex ICS-5000+ EG Eluent Generator module
  - Dionex ICS-5000+ DC Detector/Chromatography module with Dionex IC Cube and high-pressure degas cartridge
  - Dionex AS-AP Autosampler
- Chromeleon Chromatography Data System

\*Dionex ICS-4000 can be used for equivalent results

### Standards and Reagents

- 18 M $\Omega$ -cm degassed deionized water
- Dionex Combined Seven Anion II Standard (Dionex P/N 057590)

### Conditions

Column:	Dionex IonSwift MAX 200 column, 0.25 $\times$ 250 mm
Eluent Source:	Dionex EGC KOH Cartridge (Capillary) with Dionex CR-ATC Continuously Regenerated Anion Trap Column (Capillary)
Gradient:	2 to 50 mM KOH
Flow Rate:	A: 0.010 mL/min B: 0.020 mL/min C: 0.025 mL/min

IC Cube Temp.: 30  $^{\circ}$ C

Compartment Temp.: 15  $^{\circ}$ C

Inj. Volume: 0.4  $\mu$ L

Detection: Suppressed conductivity, Dionex ACES 300, suppressor  
Dionex CRD 200 Carbonate Removal Device (Capillary), recycle mode,  
A: 8 mA; B: 15 mA; C: 18 mA

Background

Conductance: 0.5–0.8  $\mu$ S conductance

Noise: < 0.3 nS

System

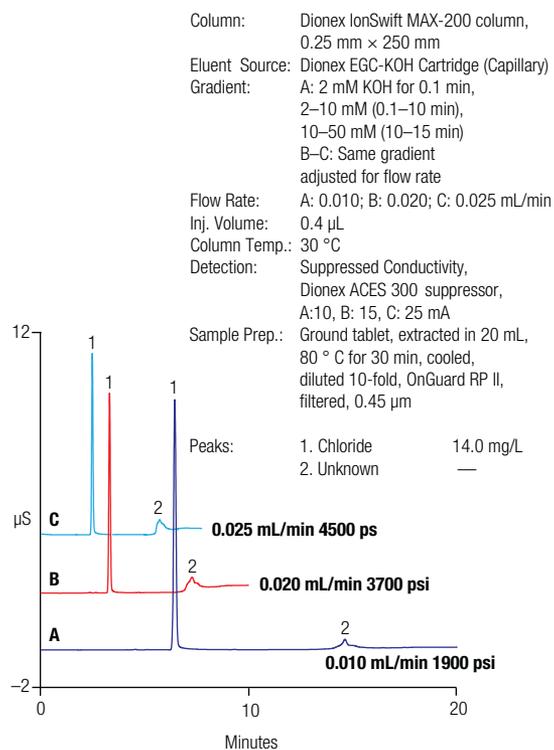
Backpressure: A: 1900 psi; B: 3700 psi, C: 4500 psi

### Analysis

Capillary Ion Chromatography

### Results

See chromatogram below.



Fast separations of counter anions in an allergy pharmaceutical drug tablet using high-pressure capillary IC.

## Ordering Information

For more information or to place an order, contact the Thermo Scientific Dionex Products office nearest you or your local distributor. Phone numbers and addresses for worldwide subsidiaries can be found in the About Us section of [www.thermoscientific.com/dionex](http://www.thermoscientific.com/dionex).

## Resources for Chromatographers

### Innovations in Ion Chromatography Resource Center

For ion analysis, nothing compares to a Thermo Scientific Dionex ion chromatography system. Explore these cutting-edge technologies and learn how to take your ion analysis to the next level.

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