New Developments in Capillary Ion Chromatography using 4 µm Columns and Charge Detection

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Overview

Purpose: New solutions highlighting the applications and benefits of high-pressure capillary ion chromatography with 4 µm particle size columns in a compact, integrated IC system are discussed. Furthermore, the benefits of a Reagent-Free™ IC system with eluent generation capabilities to achieve consistent results, day-to-day, instrument-to-instrument and operator-to-operator are presented. Advantages of suppressed conductivity with charge detection (QD) are demonstrated.

Methods: All applications were developed on an integrated, high-pressure Thermo Scientific Dionex ICS-4000 Capillary HPIC system using capillary Thermo Scientific Dionex IonPac 4 µm particle-size columns. Suppressed conductivity and the new charge detectors were used in series. Eluents were electrolytically generated, using the Reagent-Free IC system capabilities of the Dionex ICS-4000 HPIC™ system.

Results: Fast separations of biogenic amines using capillary high-pressure IC are achieved in less than 9 minutes. High-resolution separations of inorganic anions and organic acids are presented using 4 µm particle columns. More peaks can be detected in orange juice with the new charge detector.

Capillary Ion Chromatography

Ion chromatography (IC) has become one of the preferred technologies for determining ionic analytes in any type of matrix, from ultrahigh purity water, to drinking and wastewater, to fruit juices and bodily fluids. Recent developments in ion chromatography, including capillary IC, which was introduced with the Dionex ICS-5000 system, have generated great interest. In 2012, the first dedicated capillary high-pressure IC system was introduced, targeting routine analysis. In capillary IC, packed columns with internal diameters of 0.4 mm are typically used at flow rates of 10 µL/min, thus enabling continuous operation with less than 15 mL of mobile phase per day or 5.25 L per year:

Format	Standard bore	Capillary
Column i.d.	4 mm	0.4 mm
Typical Flow Rate	1.0 mL/min	10 µL/min
Typical Injection Volume	25 µL	0.4 µL
Eluent Consumption/ Waste Generation	43.2 L/month	0.432 L/month
ECG Lifetime (at 75 mM)	28 days	18 months
Mass Detection Limits	7000 fg	70 fg

Further advantages include:

- Less sample volume is required due to increased mass sensitivity. This is important for sample limited applications, e.g., the analysis of ions in biological fluids.
- The low flow rates are beneficial for interfacing with mass spectrometry, providing increased sensitivity for trace level analysis.
- Two-dimensional separations using a 4 mm inner diameter column in the first dimension and a capillary column in the second dimension allow ng/L (ppt) level detection with only 1 mL of sample and suppressed conductivity detection.

Capillary ion chromatography was built with technologies currently available for standard and microbore IC, including Reagent-Free IC with eluent generation, electrolytic suppression with conductivity detection, and electrochemical detection. Capillary IC also utilizes new technologies such as charge detection, high-pressure eluent generation, and the use of high efficiency 4 µm particle columns.

Methods

Sample Preparation
Typical sample preparation includes dilution and filtration using 0.45 µm nylon membrane filters.

Ion Chromatography

Equipment and Data Analysis

Thermo Scientific Dionex ICS-4000 Capillary HPIC System consisting of a capillary Pump, an Eluent Generator module, an IC Cube™ housing the 0.4 µL injection valve and all capillary consumables, a conductivity (CD), electrochemical (ED), or charge detector (QD), and a Dionex AS-AP autosampler.

Thermo Scientific Dionex Chromeleon Data System software, ver. 6.8 was used for instrument control and data analysis and management.

Conditions
The conditions are included in the figures.

High-Pressure Ion Chromatography

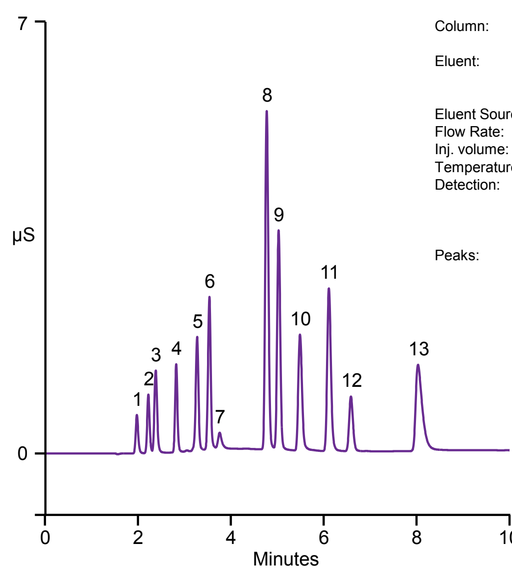
Capillary HPIC systems allow continuous operation with eluent generation at pressures up to 34 MPa (5000 psi). To extend the pressure range from 3000 to 5000 psi requires the use of HPIC components, including the pump heads, eluent genaration cartridge, and the eluent generation degasser. This enables the use of 4 µm particle size columns, which produce higher backpressures under standard conditions. The advantages of these new columns include:

- Smaller particle diameter = higher chromatographic efficiency
- Higher chromatographic efficiency =
  - faster separations at higher flow rates without sacrificing resolution

    or
  - higher resolution at standard flow rates

Figure1 shows an example for a fast separation of inorganic cations and biogenic amines in less than 9 minutes. All peaks are baseline resolved due to the high efficiency of this 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. Examples for high-resolution separations are shown in Figure 4.

FIGURE 1. Fast separation of nine inorganic cations and biogenic amines.



Column:	Dionex IonPac™ CG19-4µm/ CS19-4µm (0.4 x 250 mm)
Eluent:	9 mM MSA, gradient to 70 mM MSA at 7 min, isocratic to 7.7 min, back to 9 mM MSA at 7.8 minutes
Eluent Source:	Dionex EGC-MSA (Capillary)
Flow Rate:	0.020 mL/min
Inj. volume:	0.4 µL
Temperature:	30°C
Detection:	Suppressed Conductivity, Thermo Scientific Dionex CCES 300, AutoSuppression, recycle mode

Peaks:

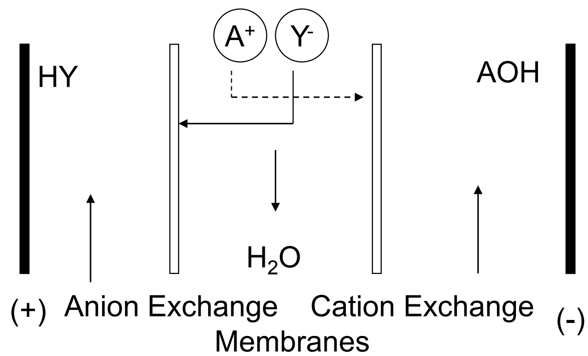
1. Lithium	0.05	mg/L
2. Sodium	0.2	
3. Ammonium	0.25	
4. Potassium	0.5	
5. Magnesium	0.25	
6. Calcium	0.5	
7. Impurity		
8. Putrescine	7.5	
9. Cadaverine	4.5	
10. Histamine	6.5	
11. Agmatine	5.0	
12. Spermine	3.0	
13. Spermidine	1.5	

NOTE: Concentrations are only approximate

Principle of Charge Detection

The charge detector uses two membranes, an anion and a cation exchange, to separate the eluent channel from two detector channels. The anode and cathode are located in the two detector channels. A constant voltage is applied to the electrodes, causing ions to migrate from the eluent channel through the membrane into the detector channels (Figure 2). When ions reach the electrodes a current proportional to the charges is generated. The resulting response is similar for the same number (molarity) of similarly charged ions independent from the degree of dissociation, thus providing universal response. Therefore, the charge detector signal is twice as strong for doubly and three times as high for triply charged ions, e.g., phosphate (Figure 5).

FIGURE 2. Schematic of a charge detector cell.



The charge detector responds to all ionic compounds and therefore is best used with hydroxide-based eluents for anion analysis or with methanesulfonic acid eluents for cation analysis, as a second detector installed in series with a suppressed conductivity detector. The charge detector removes all ions from the eluent stream and is therefore always installed last in series with other detectors. A typical set up is shown in Figures 3 and 4.

FIGURE 3. Schematic set up of a Capillary RFIC system with suppressed conductivity detection (CD) and charge detection (QD).

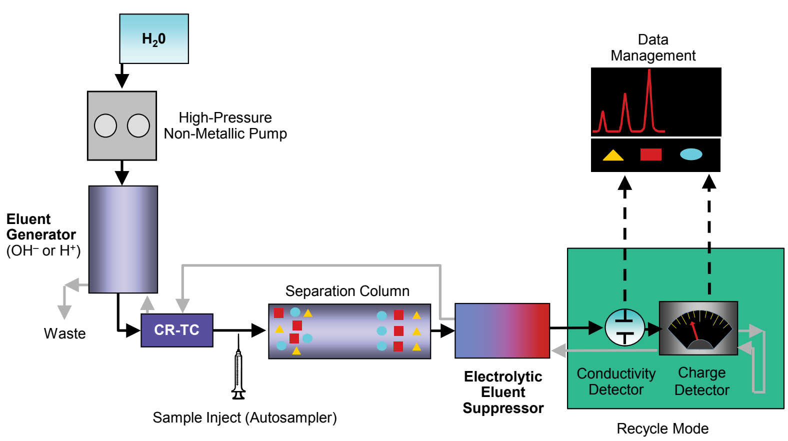


FIGURE 4. Capillary components in a Dionex ICS-4000 Capillary HPIC System.

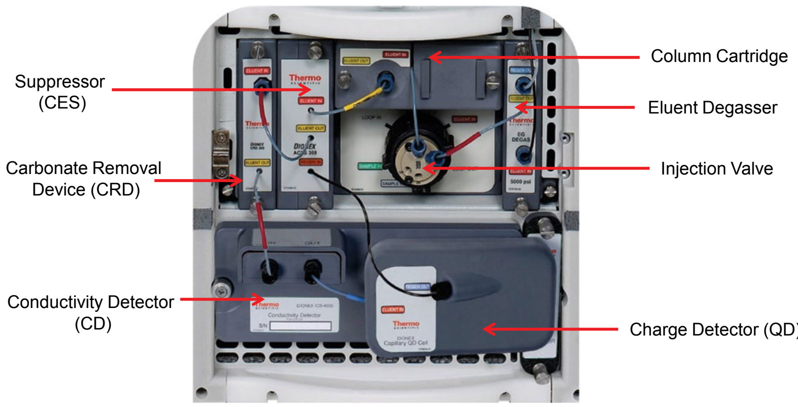
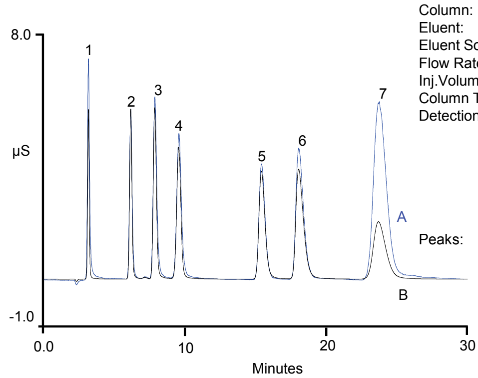


FIGURE 5. Overlaid chromatograms of a Seven Anion Standard, obtained with suppressed conductivity detection and charge detection.



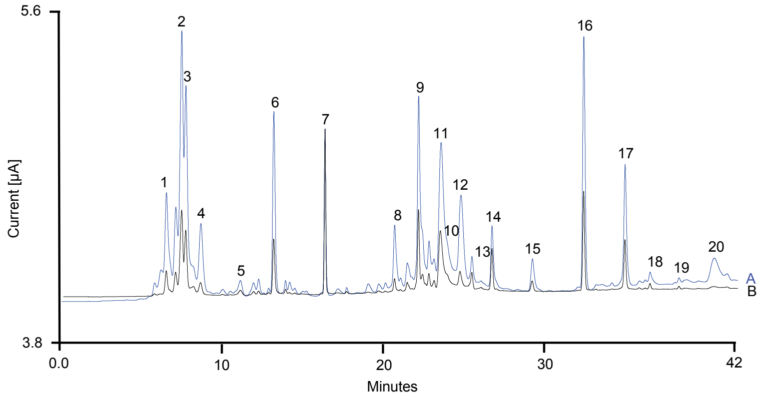
Column:	Dionex IonPac AS15-9µm, 0.4 x 250 mm
Eluent:	38 mM Potassium hydroxide
Eluent Source:	Dionex EGC-KOH (Capillary)
Flow Rate:	0.012 mL/min
Inj.Volume:	0.4 µL
Column Temp.:	30 °C
Detection:	Suppressed conductivity, Thermo Scientific Dionex ACES 300, AutoSuppression, Recycle mode
	A: Charge Detection
	B:Conductivity Detection

Peaks:		
	1. Fluoride	1.0 mg/L
	2. Chloride	2.5
	3. Nitrite	5.0
	4. Sulfate	5.0
	5. Bromide	10.0
	6. Nitrate	10.0
	7. Phosphate	15.0

Analysis of Organic Acids and Inorganic Ions in Orange Juice

The separation of organic acids and inorganic ions in an orange juice sample demonstrates the high efficiency of the Dionex IonPac AS11-HC-4µm capillary column (Figure 6). Only 0.4 µL of a 1:50 diluted, filtered sample was injected onto the column. The black trace shows the chromatogram obtained with suppressed conductivity detection and the blue trace represents the one with charge detection. The two chromatograms are scaled to the chloride peak. This example demonstrates that weakly dissociated ions such as organic acids, acetate and maleate, as well as multi-valent ions phosphate and citrate show significantly higher response with charge detection.

FIGURE 6. Determination of organic acids and inorganic anions in orange juice using a Dionex IonPac AS11-HC-4μm, 0.4 x 250 mm anion-exchange column with suppressed conductivity and charge detections.



Column:	Dionex IonPac AS11-HC-4μm Capillary (0.4 x 250 mm)	Chromatograms:	A: Charge Detection B: Conductivity Detection	
Instrument:	Dionex ICS-5000 HPIC system	Peaks:		
Eluent Source:	EGC-KOH (Capillary)			
Eluent:	1 mM KOH from 0 to 8 min, 1 mM to 30 mM KOH from 8 to 28 min, 30 mM to 60 mM KOH from 28 to 38 min, 60 mM from 38 to 42 min	1. Quinate 2. Fluoride 3. Lactate 4. Acetate	11. Carbonate 12. Maleate 13. Sulfate 14. Oxalate	
Flow Rate:	0.015 mL/min	5. Formate	15. Unknown	
Inj. Volume:	0.4 μL	6. Pyruvate	16. Phosphate	
Column Temp.:	30 °C	7. Chloride	17. Citrate	
Detection:	Suppressed conductivity, Dionex ACES™ 300 AutoSuppression, Recycle mode	8. Unknown 9. Bromide	18. cis-Aconitate 19. trans-Aconitate	
Samples:	Orange juice, 1:40 dilution with DI water	10. Nitrate	20. Unknown	

Conclusion

Newly developed solutions for capillary ion chromatography include high-pressure IC with 4 μm particle columns that enable high chromatographic resolution or fast analysis. High-pressure capillary ion chromatography is ideal for operation at higher flow rates due to the low eluent consumption. In addition, charge detection provides more information, universal detection and similar response for weakly and strongly dissociated ions.

- Capillary ion chromatography provides the benefits of low eluent consumption and low waste generation, and is ideal for analyses where sample volumes are limited.
- High pressure capillary ion chromatography using 4 μm particle-size columns provides high efficiency separations of complex samples or fast analysis.
- Reagent-Free IC systems are ideal for gradient separations, providing consistent results and eliminating the handling of corrosive acids or bases.
- The new charge detector provides increased information by detecting all ionic analytes, thus simplifying calibration and quantification.

References

For more information regarding the Dionex ICS-4000 HPIC system, charge detection, and Dionex IonPac CS19-4μm and AS11-HC-4μm columns, refer to following Thermo Fisher Scientific application notes and website.

1. Thermo Fisher Scientific website. Dionex ICS-4000 with Charge Detection, http://www.dionex.com/en-us/products/ion-chromatography/ic-rfic-systems/ics-4000/lp-111672.html
2. Thermo Fisher Scientific. *Dionex Application Note 143, Determination of Organic Acids in Fruit Juices.*
3. Thermo Scientific Dionex IonPac CS19-4μm Cation-Exchange Column, http://www.dionex.com/en-us/products/columns/ic-rfic/cation-packed/ionpac-cs19-4um/lp-111500.html
4. Thermo Scientific Dionex IonPac AS11-HC Hydroxide-Selective Anion-Exchange Column, http://www.dionex.com/en-us/products/columns/ic-rfic/hydroxide-selective-packed/ionpac-as11hc/lp-73257.html

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