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TECHNICAL NOTE 113

Practical guidance for using capillary anion chromatography

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Introduction

Capillary ion chromatography (IC), which separates ions using 0.4 mm diameter columns at 10–30 µL/min flow rates, provides increased mass sensitivity, reduces water consumption, and reduces waste generation to only ~5 L per year. The increased mass sensitivity is ideal for samples of limited amount and for trace analysis. Now smaller sample volumes—from 25 to 100 times less than those used on microbore or standard bore systems, respectively, can produce the same results.

To achieve these benefits, some additional care is required to maintain flat ends on tubing, to prevent gaps between tubing and connectors, and to prevent air bubbles from being trapped in the capillary system. The best practices described in this document help ensure that good chromatographic results are obtained as the system volumes are reduced. Additionally, a detailed comparison of standard bore and capillary Thermo Scientific™ Dionex™ Reagent-Free™ ion chromatography (RFIC™) systems is presented.

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Experimental

Equipment

- Thermo Scientific™ Dionex™ ICS-5000 capillary IC system* consisting of:
  - Thermo Scientific™ Dionex™ SP Single Pump or DP Dual Pump capillary module
  - Thermo Scientific™ Dionex™ EG Eluent Generator module
    - Capillary EGC cartridge; Thermo Scientific™ Dionex™ EGC-KOH (Capillary) (P/N 072076);
    - Capillary Continuously-Regenerated Anion Trap Column, Thermo Scientific™ Dionex™ CR-ATC (P/N 072078)
  - DC Detector/Chromatography module
    - Thermo Scientific™ Dionex™ Capillary CD conductivity detector (P/N 072041)
    - Thermo Scientific™ Dionex™ IC Cube™ capillary module (P/N 072000)
    - Degas cartridge (P/N AAA-074459)
    - Capillary column tray
    - Thermo Scientific™ Dionex™ ACES™ 300 Anion Capillary Electrolytic Suppressor (P/N 072052)
    - Thermo Scientific™ Dionex™ CRD 200 (Capillary) Carbonate Removal Device (P/N 072054)
    - High-Pressure Valve Pod, 6-port (P/N 061947)
  - Thermo Scientific™ Dionex™ AS-AP Autosampler (P/N 074921 or 074926)

* Equivalent or improved results can be achieved using the Thermo Scientific™ Dionex™ ICS-5000+ HPIC™ system or the Thermo Scientific™ Dionex™ ICS-4000 Capillary HPIC™ system.

- Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS)
- Thermo Scientific™ Dionex™ Vial Kit, 10 mL Polystyrene with Caps and Blue Septa (P/N 074228); for ultratrace work, PTFE single injection septum (P/N 074927)
- Thermo Scientific™ Nalgene™ filter flasks for eluent and autosampler flush containers (P/N 164-0020)
- Corning® polystyrene non-treated culture flasks (Fisher Scientific, P/N 08-757-502) for low-level standards and samples

Reagents

- Thermo Scientific™ Dionex™ IC Standards
  - 1,000 mg/L Fluoride (P/N 037158)
  - 1,000 mg/L Chloride (P/N 037159)
  - 1,000 mg/L Sulfate (P/N 037160)
  - Combined Seven Anion Standard II (P/N 057590)
- SPEX CertiPrep® (Fisher Scientific, P/N AS-NO29, AS-NO39, AS-PO49, AS-CL9, AS-SO49, AS-BR9, AS-F9) or Ultra Scientific certified IC Standards (Fisher Scientific, P/N USICC001, USICC002, USICC003, USICC004, USICC005, USICC006, USICC007)

Preparing the System

Flow path

Capillary IC systems have many similarities to standard bore RFIC systems, as shown in the flow diagram in Figure 1. In RFIC systems, a dual piston pump delivers deionized water to an EGC cartridge, where the eluent is electrolytically generated inline, 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, leaving only the ions to be detected by a conductivity detector.

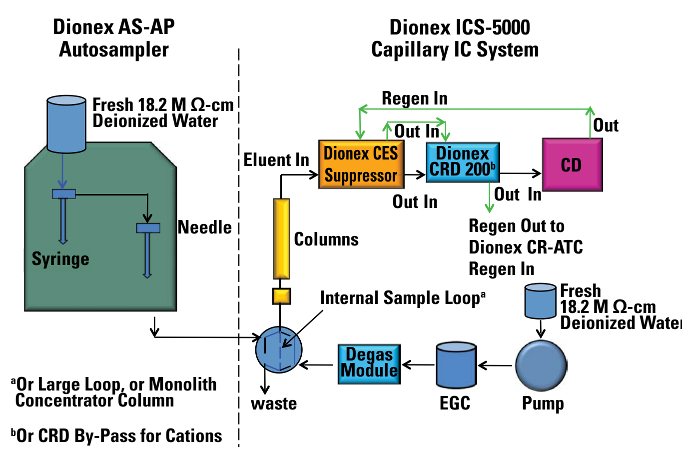

Figure 1. Flow diagram for the Dionex ICS-5000 capillary IC system.

IC Cube

In capillary IC, the flow rates are low (µL/min), so the Dionex IC Cube was designed to minimize the flow path (Figure 2) by combining most of the consumables in close proximity in the flow path and by minimizing the dead volume between them.

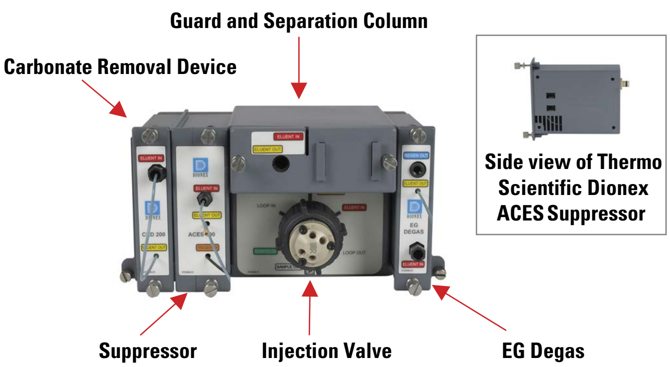

Figure 2. Components of the IC Cube module.

The Dionex IC Cube is installed directly above the capillary CD conductivity detector in the upper chamber of the Dionex ICS-5000 DC module. The Dionex IC Cube contains the EG Degas, Column Tray, Capillary Electrolytic Suppressor (Dionex CES), and Dionex CRD 200 (Capillary) carbonate removal device cartridges, plus the 4-port Rheodyne® injection valve.

Capillary Carbonate Removal Device, Dionex CRD 200 cartridge

In standard bore and microbore format anion determinations, a Dionex CRD 200 Carbonate Removal Device is recommended (but optional) to remove the carbonate peak that interferes with quantification of some adjoining analyte peaks. As the CRD increases the flow path, some analysts choose to accept a carbonate peak in their chromatography for typical-size injections of low carbonate samples rather than to install the CRD. In capillary IC, the Dionex IC Cube is also designed so that the regenerant flows through the back of the cartridges, therefore requiring that all the cartridges must be installed. Therefore for capillary IC, the Dionex CRD 200 (Capillary) cartridge or a bypass cartridge must be installed in the flow path as described in Figure 1. The CRD can also be bypassed by installing it in the Dionex IC Cube with the Eluent Out tubing connected to the Eluent In port. For large volume injections, either by direct injection or using a concentrator, a CRD is required for all formats because the carbonate peak dominates the chromatogram and interferes with quantification.

Critical practices

With the low flow rates and 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, blue connectors, and blue ferrules for all connections.

2. Minimize the void volume between the tubing and the connection.

3. Hydrate and install all cartridges in the Dionex IC Cube according to the Dionex ICS-5000 Operator's Manual.[1]

4. Diligently remove air from the system initially and after any change to the system, including turning off the pump. The pump is designed to ramp the pump speed to prevent pressure surges. This is a useful function but can interfere with attempts to stabilize the flow rate. To override this function, enter the desired flow rate, then turn the pump on, off, and on again.

5. Always keep the capillary IC system running. The capillary IC system is designed to remain on at all times. The µL/min flow rates of the capillary IC system have the advantages of low consumption of water and consumables and low waste generation, allowing the system to remain on at all times with minimal additional costs. These same low flow rates may cause longer equilibration times than standard bore systems, defeating any advantages of turning the system off. (See Reference 2 for more discussion.)[2]

6. If the system does need to be shut down, open the purge valve on the pump after turning it off to relieve the pressure in the pulse damper. Otherwise the eluent will continue to flow for several column volumes, filling the column and suppressor with water and slowing re-equilibration once the system is restarted.

For thorough, step-by-step instructions on installing the consumables in the Dionex ICS-5000 capillary IC system, including the columns, refer to the Dionex ICS-5000 Installation and Operator's Manuals.[1,3]

Fittings and tubing using blue connectors, blue ferrules and precision-cut tubing

Precision-cut tubing and proper installation of tubing and fittings are critical to achieving good chromatography, especially with the low flow rates used in capillary IC. Additionally, cleaning the connectors and ferrules by soaking them in DI water may be necessary to minimize contamination introduced into the system, particularly in trace ion analysis.

To achieve good chromatography performance in the capillary IC format, it is critical to plumb the system without voids at any of the connections, as described in the Dionex ICS-5000 Installation Manual:[3]

1. For example, to install the blue precision-cut tubing from the capillary pump mixer to the capillary Dionex EGC cartridge and then the capillary Dionex CR-ATC column, first turn the pump on and wait until liquid is flowing steadily out of the tubing.

2. Push a connector and a ferrule onto the tubing with 2 mm or more of tubing extended beyond the ferrule. [To ensure a good connection, you can extend the tubing 5 mm beyond the ferrule (Figure 3).]

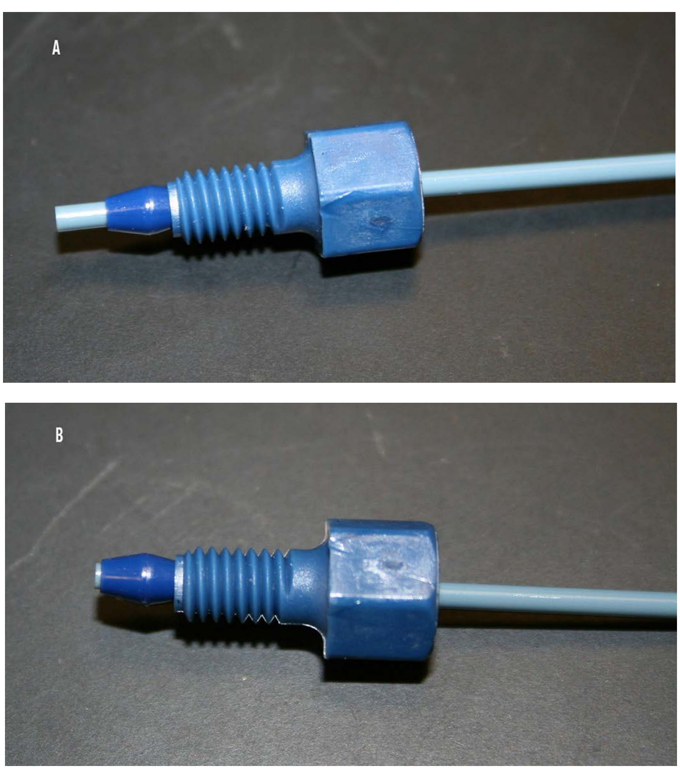

Figure 3. A) Correct and B) incorrect placement of blue connector and ferrule on precision-cut tubing.

3. Place the tube into the inlet port of the capillary Dionex EGC cartridge. While holding the tube firmly in contact with the inlet port, screw in the connector as tight as you can using your fingers. Then tighten an additional ¾ turn with a wrench.

4. Wait until liquid has filled the outlet port and is flowing out of the capillary Dionex EGC cartridge, and then connect the next piece of tubing in the same manner.

5. Follow this procedure during plumbing of all tubing connections (Figure 3) and after restarting the pump for any reason. It is critical to be diligent to prevent air from entering into the system.

6. To minimize introduced air after pump stoppage, disconnect the connection from the degas cartridge to the injection valve, turn-on the pump and check for continuous flow from the tubing, remove the next fitting and tubing in the flow path, and then re-install the previous tubing. Repeat this process through the flow path to the last fitting on the tubing connecting the CD eluent Out to the Regenerant In on the suppressor.

7. If the retention time reproducibility is poor, air bubbles may be caught in the injection valve. To flush the bubbles from the valve, temporarily increase the flow rate from the default 10 µL/min to 15– 20 µL/min.

Preparing the Dionex AS-AP Autosampler

As with all autosamplers, it is critical that the flow path be free of air bubbles to obtain good chromatography performance. Flush the flow path from the flush container to the syringe and sampling needle, and from the transfer lines to the diverter valve and injection valve. The Dionex AS-AP Autosampler needle must be aligned with the tray and the autosampler needle port, and the sample transfer line volume (TLV) from the sampling needle to the injection ports must be calibrated. Refer to the operator's manual for more information.[4]

Low-level analysis using a capillary IC system

The Dionex ICS-5000 capillary IC system provides increased mass sensitivity, so it can achieve the same results as conventional-scale systems using smaller sample volumes. For example, injection or preconcentration of a 100 µL sample on a capillary system is equivalent to a 10 mL sample on a standard 4 mm system. More trace analysis techniques using capillary IC are discussed in Dionex TN 112,[5] including:

1. Replacing the PTFE tubing to the Dionex AS-AP autosampler wash bottle and to the eluent bottle with green PEEK tubing (0.76 mm i.d.; 0.03 in i.d.)

2. Replacing the HDPE eluent and wash bottles with polystyrene Nalgene filter flask containers

3. Eliminating the eluent and wash bottles by installing a Thermo Scientific™ Dionex™ ICW-3000 Online Water Purifier system (P/N 075386) to supply clean deionized water inline without environmental contamination and without extractable contamination from the containers. The Dionex ICW-3000 Online Water Purifier system is recommended for all low µg/L to ng/L determinations.

Installing a 6-Port Pod for Large Loop and Concentrate Mode Injections

Another feature of capillary IC is that the standard 0.4 µL injection loop is internal to the injection valve, whereas most standard bore and microbore systems use a 6-port valve with a PEEK sample loop in two of the positions to load and inject the sample. To inject or concentrate sample volumes > 0.4 µL in capillary IC, remove the 4-port valve pod and replace with a 6-port valve pod (Figure 4).

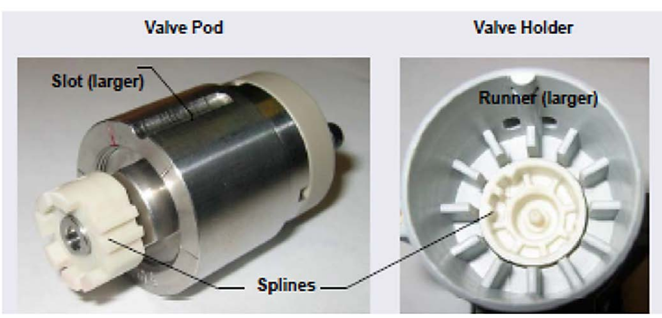

Figure 4. Injection valve pod.

Typically, the configuration using a 6-port valve pod for a Dionex ICS-5000 capillary system is installed at the factory. However, this configuration can be installed on the capillary IC system as needed. To replace the 4-port Rheodyne valve pod with a 6-port valve pod, follow the instructions in the Dionex ICS-5000 Operator's Manual.[1]

Direct Large Loop Injections

Typically an auxiliary pump and meters of tubing are used to load 1000 to 5000 µL volumes for large loop injections on standard bore and microbore formats. In capillary IC, an auxiliary pump is no longer needed; the capillary-size large volumes (2.5–250 µL) are easily loaded using the Dionex AS-AP autosampler. For large loop direct injections using capillary IC:

1. You must use a precision-cut sample loop. Install the sample loop into ports 1 and 4 of the 6-port injection valve according to the instructions in the *Fittings and Tubing* section (Figure 3).

2. Dionex AS-AP autosampler specifics, summarized from the operator's manual, for all large loop injections on all formats:

   a Instrument Configuration: On the *Options* page for the Dionex AS-AP autosampler, enter the volume you intend to load into the *Loop Size* field.

   b. Instrument Method Wizard or Program Wizard: On the *Sampler Options* page: Select *PushSeqFull* or *PushFull* in the Inject Mode field and *Both* for *Injection Wash Mode* field.

   c. Sequence: Enter the injection volume in the sequence. For *PushSeqFull* and *PushFull* modes, the injection volume must match the injection loop volume entered in the Instrument Configuration. If the two values are different, the sequence will not start.

Large loop injections can also be performed in pull mode, where the sample is pulled through the transfer line into the loop from the injection port normally used for waste. The sample is never touched by the autosampler needle. To use this method, reconfigure the tubing according to the Dionex AS-AP autosampler product manual and rename all commands described in the previous section from push to pull mode.4

Large volume injections using a monolith concentrator column

To concentrate a large volume injection on standard bore and microbore systems, 4 mm and 2 mm packed-particle ion-exchange columns are used to retain and concentrate the sample ions. In capillary IC, capillary-size monolith ion-exchange columns are used (Figure 5). These columns are produced in flexible PEEK tubing, resulting in a flexible concentrator column that is easily mounted on the 6-port injection valve (Figure 6). The Thermo Scientific™ Dionex™ IonSwift™ Monolith Anion Concentrator (MAC) columns are designed with very low void volume and low backpressure to work with high-purity capillary IC applications. Additionally, the Dionex IonSwift MAC concentrator columns use non-sulfonated resin chemistry to minimize baseline sulfate contamination. The monolith concentrator column must be installed without any voids between the tubing and port. Additionally, these monolith columns require conditioning prior to use.

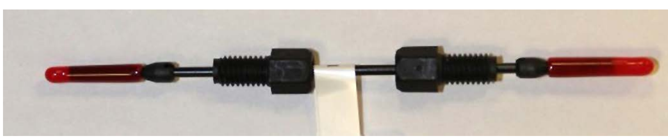

Figure 5. Dionex IonSwift concentrator column.

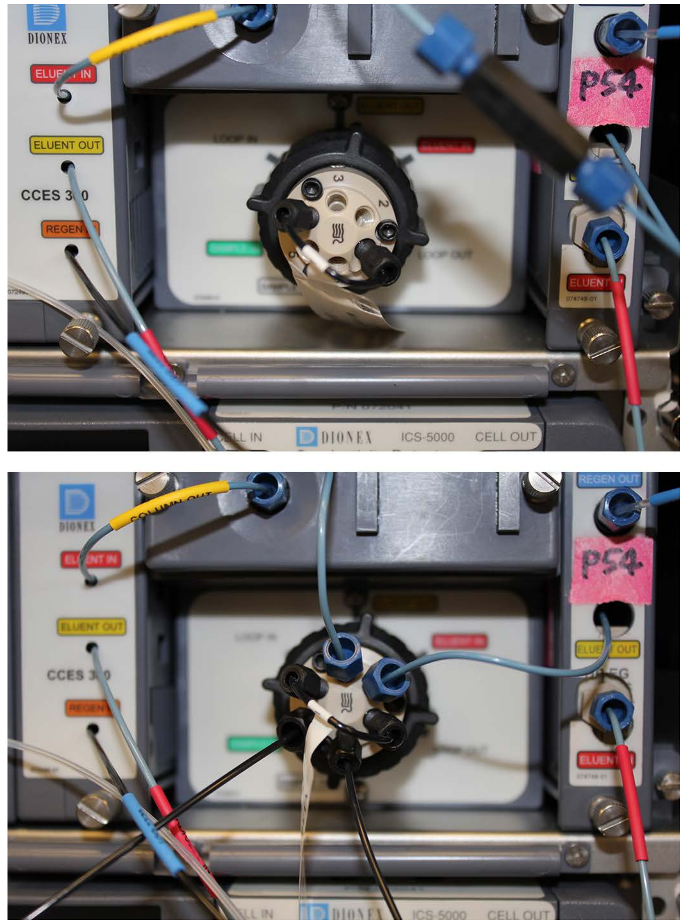

Figure 6. Dionex IonSwift concentrator column installed in ports 1 (top) and 4 (bottom) in the Dionex IC Cube 6-port valve.

1. Install the monolith concentrator column (Dionex IonSwift MAC-100 column) into ports 1 and 4 of the injection valve according to the instructions in the Fittings and Tubing section and the product manuals[6,7] (Figure 6).

2. Condition the concentrator column according to the instructions shipped with the column.

3. Dionex AS-AP autosampler specifics, summarized from the operator's manual,4 for concentration of all large loop volumes on all formats. There are exceptions for capillary IC to achieve the highest reproducibility when concentrating a large volume. This is achieved by programming a partial loop injection of a very large volume delivered at low syringe speeds needed for the capillary concentrator column to efficiently retain the anions.

   a. Instrument Configuration: On the Options page for the autosampler, enter the volume you intend to concentrate into the Loop Size field. For capillary IC, to program a partial loop injection onto the concentrator column, enter a large volume, such as 800 µL, rather than the volume to be concentrated.

b. Instrument Method Wizard or Program Wizard: On the *Sampler Options* page: Select *PushSeqConcentrate* or *PushConcentrate* in the *Inject Mode* field, *Both* for *Injection Wash Mode* field. For capillary IC, to program a partial loop injection onto the concentration column, enter 2.0 (µL/s) for the *Draw* and *Dispense* speeds (syringe) and *PushSeqPartial* or *PushPartial* in the *Inject Mode* field, *Both* for the *Injection Wash Mode* field, 2 (µL/s) for *Draw* and *Dispense* Speeds, *PushSeqPartial* or *PushPartial* in the *Inject Mode* field, 100 (µL) for *Cut Volume* field. *Loop Wash* factor should be unavailable.

c. Sequence: Enter the sample volume to be concentrated.

Results and discussion

The benefits of mass sensitivity for capillary ion chromatography have been previously demonstrated in TN 90 by running experiments in series. The author demonstrated that similar peak responses can be achieved on 4 mm, 2 mm, and 0.4 mm (capillary) columns by injecting volumes adjusted for the column dimensions.[7]

Comparable injection volume is proportional to the square of the column radius (r^2). For example, a comparable injection on a 4 mm (r = 2) standard bore column relative to a 0.4 µL injection on the 0.4 mm capillary column (r = 0.2) would be 40 µL on a 4 mm format column

$[(2 \times 2) / (0.2 \times 0.2)] \times 0.4\ \mu L = 40\ \mu L$

on a 4 mm format column.

Direct comparison using simultaneous injections

To directly compare the peak responses between injections on microbore columns and capillary columns, the same standard was simultaneously injected on both systems and separated with the same eluent concentration. Anions were separated on Dionex IonPac AS19 columns (Figure 7). Only the sample injection volumes and flow rates were adjusted (as previously described) to provide a comparable mass injection (Table 1).

Column:	Dionex IonPac AG19, AS19, A) 2 mm; B) Capillary, 0.4 mm
Eluent Source:	A. Dionex EGC III KOH; B. Dionex EGC-KOH (Capillary)
Eluent:	20 mM KOH
Flow Rate:	A. 0.25 mL/min; B. 10 µL/min
Column Temp.:	30°C
Detection:	Suppressed conductivity, A. Dionex ASRS 300 suppressor B. Dionex ACES 300 suppressor
Inj. Volume:	A. 10 µL; B. 0.4 µL

Peaks:		mg/L		
	1. Fluoride	3	6. Chlorate	25
	2. Chlorite	10	7. Bromide	25
	3. Bromate	20	8. Nitrate	25
	4. Chloride	6	9. Carbonate	—
	5. Nitrite	15	10. Sulfate	30

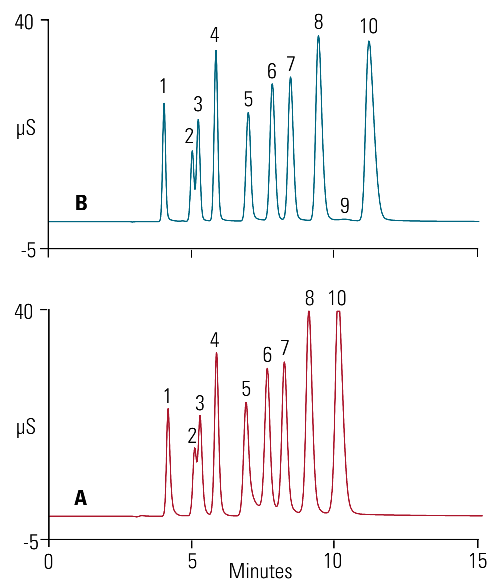

Figure 7. Comparison of the same anion standard simultaneously injected on A) 2 mm microbore and B) 0.4 mm capillary columns.

Table 1. Adjustments to injection volume and flow rate.

Column Type	Column Format		Injection Volume (µL)	Flow Rate (mL/min)
Dionex IonPac AS19	Capillary	0.4 mm	0.4	0.01
	Microbore	2 mm	10	0.25

The chromatograms show comparable results with only minor differences for both formats, A—microbore, B—capillary. However, carbonate was evident as a small peak and sulfate eluted about a minute later on the capillary Dionex IonPac AS19 column (Figure 7, Chromatogram B) than on the microbore column (Figure 7, Chromatogram A).

In contrast, sub-µL injections can only be detected on a capillary system. These lower volume requirements result in significantly lower injection volumes for trace ion analysis. A 20 µL large loop injection on a capillary format column is comparable to a 2 mL large loop injection on a 4 mm format column. Similarly in concentration mode, 200 µL on a capillary format is comparable to 20 mL on the 4 mm format.

Direct injection, large loop

The most common methods to determine trace ions use either direct injection of a large sample volume or loading on a concentrator column. Direct injections are typically used to determine low µg/L (ppb) concentrations of ions with capillary IC as well as with standard and microbore formats. The most significant differences are the sample loading method and the sample injection. For capillary IC, the 2.5–250 µL sample is loaded into a precision-cut sample loop by the Dionex AS-AP autosampler without using a very large sample loop, an auxiliary pump, and the meters of tubing required to plumb the pump. In this example, a 10 µL loop of a ~1 µg/L standard is loaded by the Dionex AS-AP autosampler onto a Dionex IonPac AS19 column set (Figure 8). The inorganic anions are separated using a gradient from 14 to 45 mM KOH at 10 µL/min and are eluted from the column within 20 min. This injection volume is equivalent to 1 mL and 250 µL for standard and microbore formats, respectively.

Calibrating using direct large sample injections

Calibrating for large loop injections is performed in the standard way, using standards of different concentrations to calibrate different levels. Typically a calibration curve of peak area response versus concentration is established using single or multiple injections of 3 to 5 standards covering a concentration range from slightly above the limit of quantification to slightly above the expected highest unknown sample concentration. In this study, the anion results using replicate injections of the three volumes were linear, with coefficients of determination (r^2) from > 0.999.

Column:	Dionex IonPac AG19, AS19, capillary, 0.4 × 250 mm
Eluent Source:	Dionex EGC-KOH capillary
Eluent:	14 mM KOH from 0 to 7 min, 14–45 mM from 7 to 25 min, 14 mM from 25.1 to 35 min
Flow Rate:	10 µL/min
Column Temp.:	30°C
Inj. Volume:	10 µL
Detection:	Suppressed conductivity, Dionex ACES 300, Dionex CRD 200 capillary
Vial Septa:	Blue Septa

Peaks:		µg/L		
	1. Fluoride	0.2	6. Bromide	1.0
	2. Acetate	—	7. Nitrate	1.0
	3. Formate	—	8. Carbonate	—
	4. Chloride	1.0	9. Sulfate	1.0
	5. Nitrite	1.0	10. Phosphate	2.0

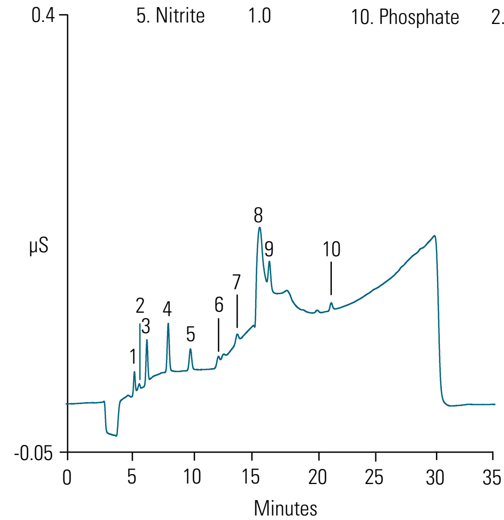

Figure 8. A large-loop direct injection of a 1:100,000 diluted Dionex Seven Anion II Standard onto a capillary Dionex IonPac AS19 column set.A) 2 mm microbore and B) 0.4 mm capillary columns.

Large volume injection by concentration

For larger injection volumes, it is more practical and efficient to concentrate the ions onto an ion-exchange concentrator column prior to injection. This approach is the same for all column formats, except monolith ion-exchange columns are used for concentrating on capillary IC instead of packed-bead ion-exchange columns used for the larger format columns. These ion exchange monoliths columns with very low void volumes, such as the Dionex IonSwift MAC-100 column, are optimized for capillary IC. As the sample is loaded onto the concentrator column, the ions are retained and the water matrix of sample passes to waste. The valve is switched, and the eluent elutes the ions to the guard and separator columns. The elimination of the water matrix as the eluent flows through valve and through concentrator column from the opposite side, peak response by a factor of 3–5. In this example, only 200 µL of a 10–25 ng/L anion standard is concentrated by the Dionex AS-AP autosampler onto a Dionex IonSwift MAC-100 concentrator column (Figure 9). The

concentrated ions are eluted from the concentrator and separated on a Dionex IonPac AS15 capillary anion-exchange column set at 12 µL/min using electrolytically generated 38 mM KOH. This injection volume is equivalent to 20 mL and
5 mL injected on standard bore and microbore columns, respectively. Trace ion determinations at double digit ng/L concentrations, as shown in this example, are challenging. To reach these low concentrations and low background contamination, the Dionex ICW-3000 Online Water Purifier feeds deionized water inline directly to the eluent pump and autosampler without any intermediate containers, and to the suppressor in external water mode as the regenerant.

Column: Dionex IonPac AG15, AS15, capillary, 0.4 × 250 mm
Eluent Source: Dionex EGC-KOH (capillary), ICW-3000 Online Water Purifier
Eluent: 38 mM KOH
Flow Rate: 12 µL/min
Column Temp.: 30°C
Detection: Suppressed conductivity, Dionex ACES 300, external water, ICW-3000 Online Water Purifier, Dionex CRD 200 capillary
Inj. Volume: 200 µL
Concentrator: Dionex IonSwift MAC-100
Vial Septa: PTFE Single Injection Septum

Peaks:				
1. Fluoride	11 ng/L		5. Nitrite	24
2. Acetate	—		6. Sulfate	22
3. Chloride	26		7. Oxalate	—
4. Carbonate	—		8. Nitrate	26

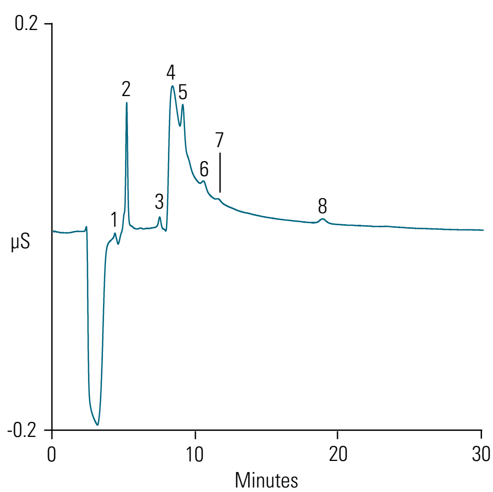

Figure 9. Trace anions in a water blank injection determined by concentrating 200 µL on a Dionex IonSwift MAC-100 concentrator column.

Calibrating using concentrate mode

To calibrate using a concentrator column, different volumes of the same working standard can be concentrated from the same vial, such as 50, 100, 200 µL of ~0.5 µg/L mixed standard. Using this method, the calibration validates both the linearity of the peak responses and the efficiency of the concentrator column. However, this method will have systematic bias if there is any error in the concentration of the working standard, so special care should be taken to ensure that its concentration is accurate. The anion results using replicate injections of the three volumes were linear with similar coefficients of determination (r^2) as found using the large loop injections.

Precautions

Trace and ultratrace ion analyses (low µg/L to ng/L) in ultrapure water are challenging analytical techniques that require considerable effort, time, and patience. Many analysts experienced in trace ion analysis may initially spend several weeks to first reduce the baseline contamination to an acceptable level, then diligence to stabilize the baseline contamination, before finally starting to analyze samples. To the inexperienced analyst, trace analysis can be more challenging.

Conclusion

Capillary IC has been shown to produce results similar to those obtained using conventional-scale IC, while offering additional benefits:

- Higher mass sensitivity, which enables use of smaller sample volumes
- Greatly reduced eluent consumption and waste generation
- More convenient sample preconcentration, because no sampling pump is required for large volume samples
- Increased convenience, because the system is always ready for injections
- Time and cost savings, because the system requires much less calibration and equilibration[2]
- Additional information about using capillary IC for trace analysis can be found in TN 112.[5]

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