

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

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Overview

Purpose: This work reports the development and the advantages of a walk up capillary IC system using high pressure to provide ultrafast separations of inorganic anions and cations relevant to the pharmaceutical industry.

Methods: Counterions relevant to the pharmaceutical industry are separated by capillary ion-exchange chromatography and detected by suppressed conductivity on a capillary IC system using Thermo Scientific Dionex Capillary Electrolytic Suppressors (CES™).

Results: This work demonstrates the advantages of using a capillary IC system that is Always On, Always Ready for analysis.

Introduction

Ion chromatography (IC) with suppressed conductivity detection is a well-established technique for the determination of inorganic and organic ions in pharmaceuticals. The recent development of high-pressure capillary IC brings additional advantages for the analysis of inorganic ions. Because the eluent consumption is very small, capillary IC systems can be operated continuously and therefore are always on and always ready for analysis, redefining the workflow for IC, and improving method performance. Capillary IC systems offer improved compatibility with applications where sample amount is limited. The operation of capillary IC at higher pressures and higher flow rates improves the separation efficiency and/or speed. 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 On, Always Ready system brings to IC analysis.

Methods

Sample Preparation

Samples:

- Metaformin HCl, Fexofenadine HCl tablets
- Naproxen sodium, Atorvastatin calcium tablets

The tablets were ground, dissolved in water at 45 °C, filtered using a Thermo Scientific Dionex OnGuard™ RP cartridge and 0.5 µm IC filter, and diluted 1:10 prior to analysis.

Liquid Chromatography

Equipment and Data Analysis

Thermo Scientific Dionex ICS-5000 Reagent-Free™ Capillary IC system consisting of:

- DP Dual isocratic capillary pump
- DC Detector and Chromatography Module
 - IC Cube™ capillary module compartment
 - CD Capillary Conductivity Detector for Anions and Cations
- EG Eluent Generator
- AS-AP Autosampler with diverter valve
- Thermo Scientific Dionex Chromeleon™ Chromatography Data System

Conditions

All analytes were separated with ion-exchange chromatography and detected by suppressed conductivity using CES suppressors, as described in the figures.

Figure 1 shows the typical workflow for a continuously operated IC system. The system is equilibrated and ready to run samples. A check standard is run to verify system performance followed by the samples. This mode of operation lends itself ideally to environments where multiple users require the use of IC and method performance is of high importance.

FIGURE 1. Always On, Always Ready – Walk-Up IC workflow.



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Results

Walk-Up IC Systems:

Operating an IC system in the Always On, Always Ready mode brings many advantages to IC:

- It simplifies and speeds up the analysis by eliminating time consuming and error prone steps such as manual eluent preparation, startup and equilibration time.
- The system is more stable in terms of noise and detector response.
- Enhanced stability saves time as fewer calibration sequences are required and the system can be quickly verified for system performance by just running a check standard.
- Lends itself to multiuser operation as it allows an operator to walk up to the system and obtain results with minimal training.
- Decreases preventive maintenance and down time.

Advantages:

The advantages of operating a capillary IC system in the Always On, Always Ready mode are:

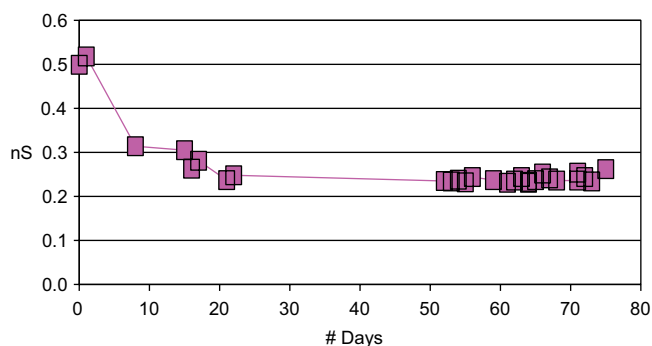
1. Consumes only 15 mL/day of deionized water, equating to 5.2 L/year.
2. Reagent-Free IC (RFIC™) system technology plus capillary IC provides ease of use, high performance, and gradient eluent generation of potassium hydroxide.
3. More cost effective: The Thermo Scientific Dionex Eluent Generation Cartridge (EGC) lasts for 18 months of continuous operation under standard operating conditions, lowering the overall cost of ownership.
4. Higher eluent concentrations: The capillary eluent generator cartridge can generate up to 200 mM concentration, adding method flexibility and robustness.
5. Higher pressures and faster flow rates: The capillary-format Dionex EGC is compatible with pressures up to 5000 psi, facilitating shorter run times, increased productivity, and faster turnaround of results.

System Performance

Peak-to-Peak Noise

Figure 2 illustrates the stability of a capillary IC system running in continuous operation mode.

FIGURE 2. Peak-to-peak noise of capillary IC using Thermo Scientific Dionex IonPac™ CS16 cation columns over 80 days: Conditions—Eluent: 30 mM methanesulfonic acid (MSA); Flow 10 μ L/min; Suppressor: Thermo Scientific Dionex CCES™ 300 Cation Electrolytic Suppressor.

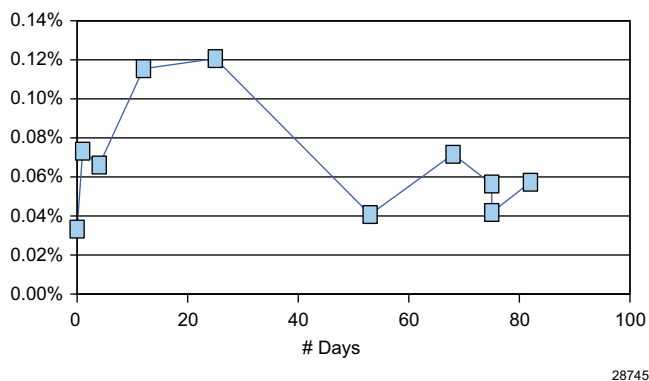


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Retention Time and Peak Area Stability

Figure 3 illustrates the retention time stability of a capillary anion IC system running in continuous operation mode. The average retention time reproducibility for anions was well below 0.15%. This level of reproducibility provides accurate peak identification due to minimal shifts in analyte peaks.

FIGURE 3. Average anion peak retention time reproducibility of Capillary IC using Dionex IonPac AS19 anion columns over 90 days: Conditions—Eluent: 20 mM KOH; Flow 10 μ L/min; Suppressor: Thermo Scientific Dionex ACES™ 300 Anion Electrolytic Suppressor.



Peak Area Stability and Linearity

Figures 4 and 5 illustrate the peak area stability of capillary anion and cation IC systems running under continuous operation mode. This level of enhanced stability saves time as fewer calibration sequences are required and the system can be quickly verified for system performance by just running a check standard. Tables 1 and 2 illustrate the linearity performance for the capillary anion and cation systems. The coefficient of linearity (r^2) ranged from 0.9993 for magnesium to 1.000 for nitrite.

FIGURE 4. Average anion peak area reproducibility.

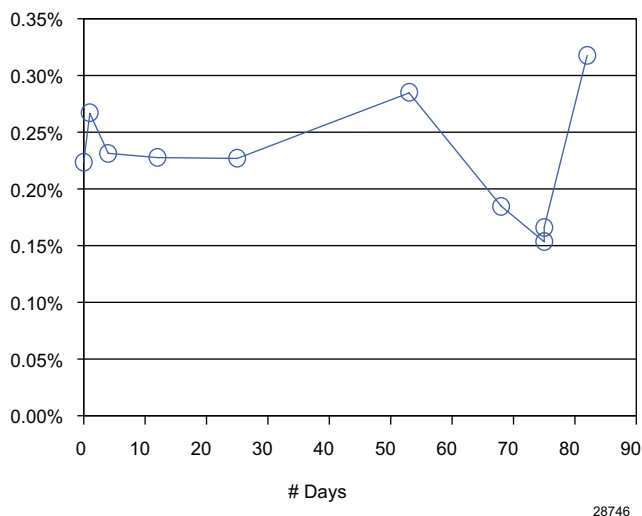


FIGURE 5. Average cation peak area reproducibility.

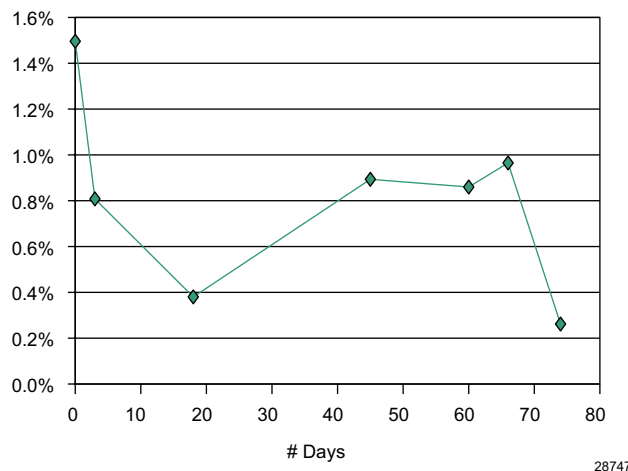


Table 1. Linearity for Anion Capillary System Using the Dionex IonPac AS19 Column

	Linear Range (µg/L)	Standard Deviation of % Residuals
Fluoride	1.5–150	0.068
Chlorite	5–500	0.97
Bromate	10–1000	1.24
Chloride	3–300	1.92
Nitrite	7.5–750	2.34
Chlorate	12.5–1250	2.41
Bromide	12.5–1250	2.62
Nitrate	12.5–1250	3.48
Sulfate	15–1500	14.0

Table 2. Linearity for Cation Capillary System Using the Dionex IonPac CS16 Column

	Linear Range (µg/L)	Standard Deviation of % Residuals
Lithium	0.5–50	0.043
Sodium	2–200	0.056
Ammonium+	2.5–250	0.093
Potassium	5–500	0.090
Magnesium	2.5–250	0.130
Calcium	5–500	0.161

High Pressure Capillary IC:

- With the introduction of high-pressure capillary IC, the system is now capable of continuous operation up to 5000 psi with RFIC system technology.
- These new capabilities allow it to take advantage of smaller particle size resin for high-resolution separations, and to accelerate the analysis by simply increasing the flow rate, resulting in higher throughput and sample turnaround.

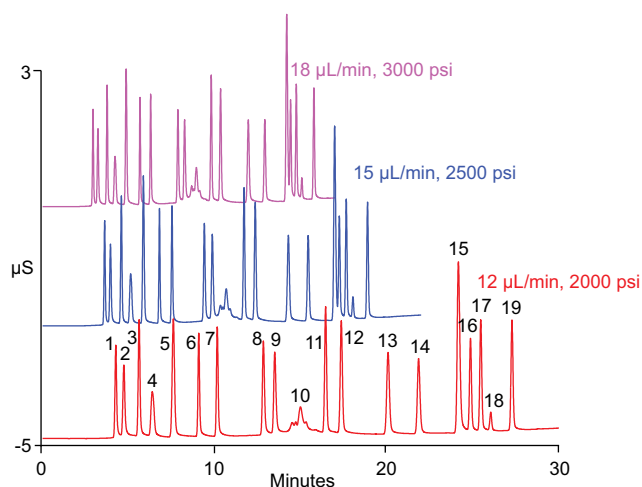
Figures 6 and 7 illustrate the use of flow rate to decrease the run time for a multi-anion and cation mixture using the capillary platform. The separation of 19 anions relevant to the pharmaceutical industry can be cut in half by increasing the flow rate from 10 to 18 µL/min.

Similar acceleration can be achieved for the analysis of cations. Increasing the flow from 10 to 30 µL/min, the run time can be reduced from 18 min to under 6 min.

FIGURE 6. Fast anion determinations of 19 anions standard using high-pressure capillary IC.

Column: Thermo Scientific Dionex IonSwift™ MAX-100G, MAX-100, 0.25 mm
 Eluent Source: Dionex EGC-KOH Capillary, KOH gradient
 Flow Rates: 12, 15, and 18 µL/min
 Inj. Volume: 0.4 µL
 Column Temp.: 35 °C
 Detection: Suppressed conductivity, Dionex ACES 300

Peaks: 1. Fluoride 11. Sulfate
 2. Acetate 12. Oxalate
 3. Formate 13. Tungstate
 4. Butyrate 14. Phosphate
 5. Galacturonate 15. Chromate
 6. Chloride 16. Citrate
 7. Nitrite 17. Isocitrate
 8. Bromide 18. *cis*-Aconitate
 9. Nitrate 19. *trans*-Aconitate
 10. Carbonate

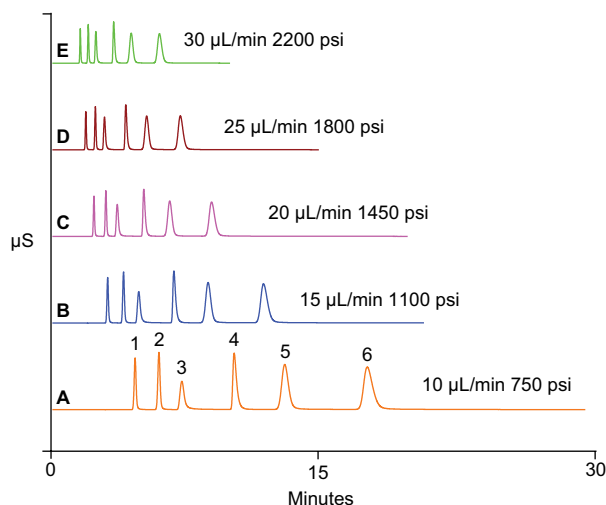


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FIGURE 7. Fast cation determinations by high pressure capillary IC.

Column: Dionex IonPac CS16 Capillary, 0.5 mm × 250 mm
 Eluent Source: Dionex EGC-MSA Capillary
 Eluent: 30 mM MSA

Peaks: 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



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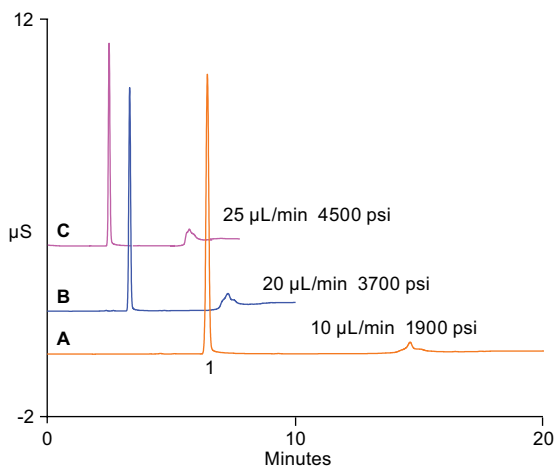
Analysis of Counterions in Pharmaceutical Products

Pharmaceutical compounds are often charged compounds with a counterion. This counterion is an important part of the formula weight and impacts the effective concentration of the active pharmaceutical ingredient (API). Additionally, the regulatory agencies require that pharmaceutical companies determine the composition of all ingredients.

Figures 8–11 illustrate the potential of capillary IC for the sensitive and selective determination of counterions in pharmaceutical formulation using ion exchange with suppressed conductivity detection. High-pressure capabilities of the capillary format allow the use of higher flow rates to reduce run times and increase sample throughput and result turnaround.

FIGURE 8. Fast IC: Counteranions in Allegra at different flow rates on Dionex IonSwift MAX-200 column.

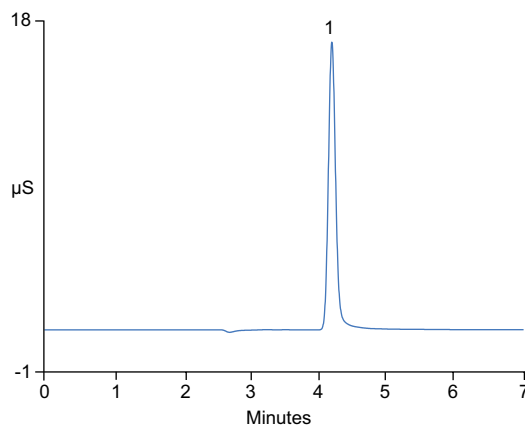
Column:	Dionex IonSwift MAX-200, 0.25 × 250 mm	Detection:	Suppressed conductivity, Dionex ACES 300
Eluent Source:	Dionex EGC-KOH Capillary	Sample:	3.66 mg/mL of 100 mg tablet in water
Gradient:	2 to 50 mM KOH	Sample Prep.:	Filtered, Dionex OnGuard™ RP, 1:10 dilution
Flow Rate:	A: 10 µL/min B: 20 µL/min C: 25 µL/min		
Column Temp.:	30 °C	Peak:	1. Chloride
Inj. Volume:	0.4 µL		mg/L µg/tablet 16.6 453



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FIGURE 9. Counterion determinations in a metformin HCl tablet by capillary IC.

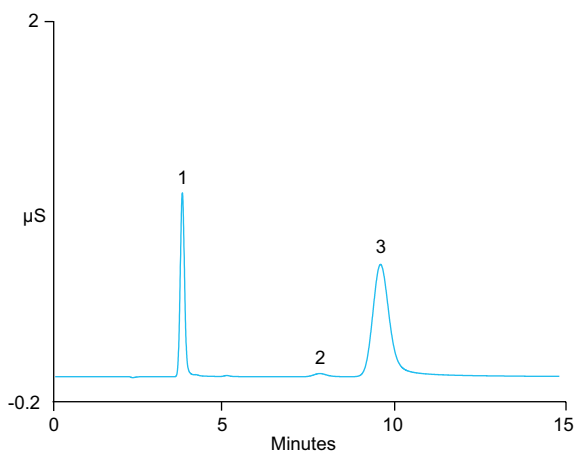
Column:	Dionex IonPac AS19 Capillary, 0.4 × 250 mm	Inj. Volume:	0.4 µL
Eluent Source:	Dionex EGC-KOH Capillary	Detection:	Suppressed conductivity, Dionex ACES 300
Eluent:	40 mM KOH	Sample:	3.66 mg/mL of 100 mg tablet in water
Flow Rate:	10 µL/min	Sample Prep.:	Filtered, Dionex OnGuard RP, 1:10 dilution
Column Temp.:	30 °C		
Inj. Volume:	0.4 µL		
		Peak:	1. Chloride
			mg/L µg/tablet 16.6 453



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FIGURE 10. Counterion determinations in a atorvastatin calcium tablet by capillary IC.

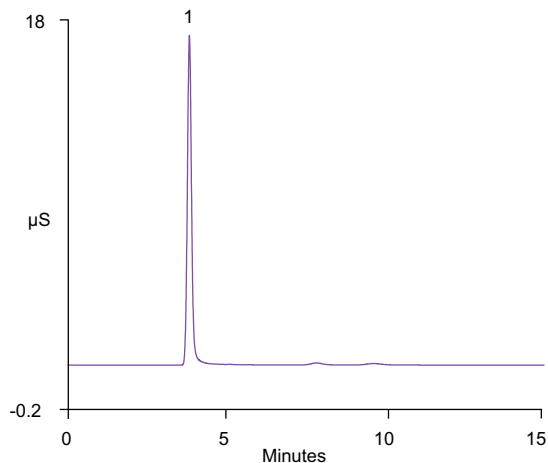
Column:	Dionex IonPac CS12A, Capillary, 0.4 × 250 mm	Sample Prep.:	Filtered, Dionex OnGuard RP, 1:10 dilution
Eluent Source:	Dionex EGC-MSA Capillary		
Gradient:	20 mM MSA		
Flow Rate:	10 µL/min	Peaks:	1. Sodium mg/L µg/tablet 0.147 1.13
Column Temp.:	30 °C		2. Magnesium 0.705 5.46
Inj. Volume:	0.4 µL		3. Calcium 3.59 27.8
Detection:	Suppressed conductivity, Dionex CCES 300,		
Sample:	1.29 mg/mL of 10 mg tablet in water		



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FIGURE 11. Counterion determinations in a naproxen sodium tablet by capillary IC.

Column:	Dionex IonPac CS12A-8 μm Capillary, 0.4 \times 250 mm	Detection:	Suppressed conductivity, Dionex CCEs 300
Eluent Source:	Dionex EGC-MSA Capillary	Sample:	1.21 mg/mL of 200 mg tablet in water
Gradient:	20 mM MSA	Sample Prep.:	Filtered, Dionex OnGuard RP, 1:10 dilution
Flow Rate:	10 $\mu\text{L}/\text{min}$		
Column Temp.:	30 $^{\circ}\text{C}$		
Inj. Volume:	0.4 μL	Peaks:	1. Sodium 9.14 1510



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Conclusion

- Capillary IC with RFIC system technology redefines the workflow for ion chromatography by simplifying how IC is utilized in laboratories and speeding-up the overall process from sample to result, saving time and lowering the overall cost of ownership.
- The Always On, Always Ready mode of operation improves method performance of the ion chromatograph, providing high accuracy, reproducibility, sensitivity, and confidence in results.
- High pressure IC in the capillary format provides additional benefits of Fast IC and high-resolution ion analysis
- Ion exchange with suppressed conductivity detection provides a highly sensitive and selective detection mode for the analysis of counter ions in Pharmaceutical formulations.

References

1. Thermo Fisher Scientific. *Dionex Application Note 106, Ion Chromatography in the Pharmaceutical Industry.*
2. Thermo Fisher Scientific. *Dionex Application Note 164, Assay for Citrate and Phosphate in Pharmaceutical Formulations Using Ion Chromatography.*

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